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Filamentous Bacterial Viruses

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BACKGROUND

Introduction

Filamentous bacterial viruses (FV) are long deoxyribonucleoproteins about 5.5 nm in diameter. Their deoxyribonucleic acid (DNA) is a single-stranded ring containing fewer than 10,000 nucleotides. Their hosts are gram-negative bacteria. They adsorb at the tips of the threadlike bacterial appendages known as the sex pili, which are involved in transmission of plasmids from one bacterium to another. At least two species of these viruses can be distinguished, corresponding to the two known types of sex pili.

Unlike other bacterial viruses, FV are released from growing and dividing cells without markedly harming the cells. This symbiotic behavior requires the definition of a new type of bacteria, bacteria which have the property of releasing infectious filamentous nucleoprotein particles. These carrier strains of bacteria can be subcultured and stored as other strains, although they are somewhat unstable, and will continue to release virus particles into the medium.

Partly because of this nonlytic mode of release, it is possible to grow high-titer cultures of the virus. A raw titer of 5×10^{12} plaque-forming units/ml is not uncommon. Since this is almost 150 mg of infective virus per liter of raw culture, and the plating efficiency is about 50%, a yield of 50 to 100 mg of purified virions per liter of culture is quite possible.

The properties of FV make them valuable for studying several questions of interest. Some of these questions can also be studied with the spherical single-stranded DNA viruses such as ϕX174, but in certain respects the FV have unique advantages.

(i) These viruses are among the smallest viruses known, so that it is feasible to define all of the virus functions at the molecular level and to consider the interaction of the various functions: the "systems" aspect of molecular biology.

(ii) In particular, the small genome size makes it relatively easy to study the mechanics, enzymology, and regulation of DNA replication and recombination of FV.

(iii) The purified virions themselves are a plentiful source of homogeneous stable deoxyribonucleoprotein for physical studies. This type of bacterial virus is the only bacterial virus which has been crystallized in a form suitable for structure analysis with X-ray diffraction techniques. Since bacterial viruses are particularly convenient for genetic studies, this is a good system in which to analyze the effect of mutation on virus structure and morphopoiesis. Analysis of the structure of the virion will provide information which may increase our understanding of deoxyribonucleoproteins in general and nucleohistone in particular.

(iv) Study of the unusual method of virus penetration and release may lead to useful knowledge about the nature of the bacterial cell envelope. In fact, the cell membrane itself may be a morphopoietic factor in the assembly of the virion.

(v) Infection by FV alters the metabolism of the bacterial cell without killing the cell. One may hope to learn more about the bacterial cell by studying the nature of these perturbations. Since useful information has been obtained by measuring parameters of bacterial growth after shift of temperature or medium, so may useful information be obtained by measuring such parameters after FV infection.

(vi) Certain aspects of the interaction of virus and host suggest that this system may have some relevance as a model for infection of eucaryotic cells by animal viruses, in particular oncogenic viruses.

The field of FV has been covered in three other reviews (86, 142, 151). Although we sought to avoid duplicating these reviews in our study, for completeness we tried to cite every paper in the field. Occasionally, we will refer to work on the spherical, single-stranded DNA phages ϕX174 and S13, since some aspects of FV replication are similar to the better-studied ϕX174 system. Several reviews on spherical, single-stranded DNA phage are available (142, 151, 175–178, 185).

In this review, we will often interpret experiments in terms of our current visualization of bacterial molecular physiology. We realize that many of these models may be incorrect. But if we provoke some workers sufficiently to cause them to do experiments to disprove our models, the review will have had some value.

History

In 1960, several bacterial viruses specific for male strains of bacteria were reported (118). Among these viruses was one, called f1, which was not studied further at the time because the virus

TABLE 1. *Ubiquity of FV*

Genus[a]	Species[a]	Strain	Host	Source	Reference
Inovirus	*andreios* (Ff)	fd[b]	*Escherichia coli*	Heidelberg, Germany	125
		f1	*E. coli*	New York, United States	118, 205
		M-13[c]	*E. coli*	Munich, Germany	89
		ZJ/2	*E. coli*	Pangbourne, England	21, 22
		Ec9	*E. coli*	Milan, Italy	59
		AE2	*E. coli*	Adelaide, Australia	140
		HR	*E. coli*	United States	95
		δA	*E. coli*	Japan	136
	Unclassified	Xf	*Xanthomonas oryzae*	Taiwan	113
Dolicho-inovirus	*colicinus* (If)	If1	*Salmonella typhimurium*	Billericay, England	115, 129
		If2	*S. typhimurium*	Croyden, England	115, 129
	Unclassified	Pf1	*Pseudomonas aeruginosa*	Japan	132, 183
Unclassified	Unclassified	Pf2	*P. aeruginosa*	Japan	132
		v6	*Vibrio parahaemolyticus*	Japan	135

[a] See Appendix for a discussion of classification.
[b] ATCC 15669 B_2.
[c] ATCC 15669 B_1.

plaques were turbid and therefore difficult to detect; most work was put into the study of the spherical ribonucleic acid (RNA) virus f2.

This report of male-specific bacterial viruses led to the isolation of male-specific viruses in several other laboratories. One of these viruses (fd) proved to contain single-stranded DNA. At the time, the only single-stranded DNA bacterial viruses known were the spherical viruses similar to φX174. During attempts to purify the supposedly spherical fd, it was found that infectivity always traveled with the filamentous particles. After repeated attempts to purify the virions free from these filaments, it was realized that the filaments were the virions. This fact was confirmed by demonstrating that fd infectivity is extremely sensitive to shear, as expected for a long, thin particle but unlike the spherical φX174 (125).

Another series of small bacterial viruses was isolated taking advantage of the fact that the nucleic acid of small viruses is infective to bacterial spheroplasts. One of these viruses (M-13) was also found to be filamentous and male-specific (89).

At the same time as fd and M-13 were being characterized, a study of f1 showed that the buoyant density of this virus in CsCl is quite different from that found for f2. Electron microscopy revealed that f1 is also filamentous (205).

Since the reports of these three FV in 1963, many other such viruses have been isolated from various sources (Table 1). The history of several of these other viruses is interesting.

Virus v6 was discovered by plating culture supernatant fluid of *Vibrio parahaemolyticus* strain K2 on strain K11; about 10^3 to 10^4 plaque-formers/ml were found (135). Similarly, virus Pf2 was detected by plating the supernatant fluid of *Pseudomonas aeruginosa* strain P28 on strain K; about 10^4 plaque-formers/ml were found (132). Thus, both v6 and Pf2 were apparently present in the carrier state when they were first isolated.

The filamentous virus HR was isolated by selecting for heat resistance and plating on various bacteria (95). Other filamentous viruses are also heat-resistant, so this is a general selection method for filamentous viruses.

Two morphologically and serologically distinct types of sex pili are known: F pili, such as those produced by bacteria harboring the sex factor F; and I pili, such as those produced by bacteria harboring the colicin factor I (129). F-specific filamentous viruses (Ff) do not adsorb to I pili, but it was reasoned that there might exist bacterial viruses which are specific for I pili. By using suitable selection techniques, I-specific filamentous viruses (If) have been isolated on *Salmonella typhimurium* (115, 129). (See Appendix for a discussion of the classification of FV.)

Ecology

Study of the interaction of a bacterial virus with its environment can be called the "ecology" of the

virus. The ecology of FV differs in several respects from that of all other bacterial viruses.

The growth curve of FV shows a rapid increase of virus after a latent period, as for other bacterial viruses. But virus titer continues to increase after the initial rise, at a constant rate of 200 to 2,000 viruses per bacterium per bacterial doubling (depending on conditions), until after the bacteria enter the stationary phase (35, 85, 95, 112, 129, 135, 136, 140; Fig. 1).

There is no killing or lysis of the host after normal infection with FV. Micromanipulation experiments showed that individual cells divide and form clones while releasing virus (88). Electron micrographs of infected bacteria suggest that cells remain physically intact after releasing virus (90; Fig. 2). There is no release of β-galactosidase from infected cells (85, 140, 162). Thus, an early definition (54) of true lysogenesis as "the condition in which virus particles are produced and secreted by their host cells without lysis of the latter," applies to FV.

Cell number (turbidity or colony formers) continues to increase in infected cultures, but the growth rate is lower than in uninfected cultures. In general, turbid-plaque strains inhibit bacterial growth least in liquid culture, and clear-plaque strains inhibit most (35, 85, 95, 129, 132, 135, 162). This reduction in growth rate is the basis of a method that is sometimes useful for determining the number of infected cells in a culture: infected cells give smaller colonies than non-infected cells. Good results with this method depend on close control of conditions such as water content of the agar plates.

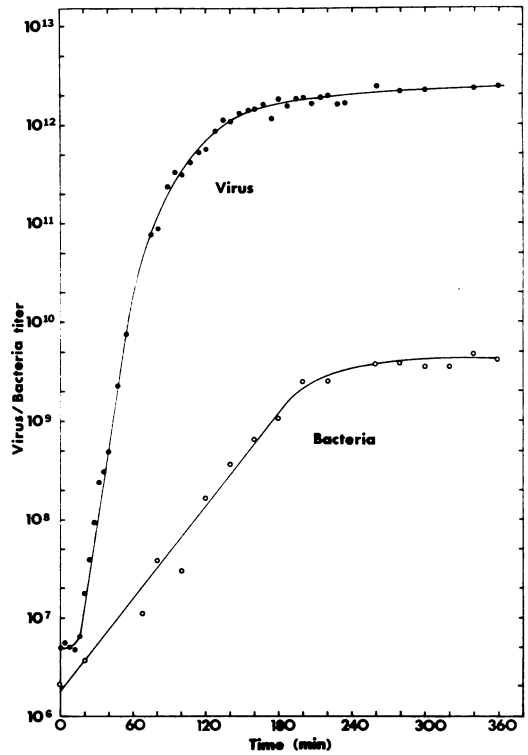

FIG. 1. *Growth curve for Ff. E. coli Hfr S26 (from A. Garen) was grown at 37 C in tryptone medium (127) with aeration. During log phase, the culture was infected with fd. Samples were withdrawn at intervals, diluted 1:100 into iced buffer, and analyzed for colony formers and plaque formers (R. L. Wiseman, unpublished data).*

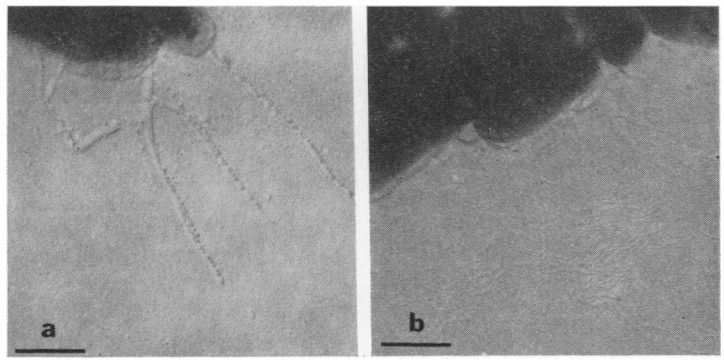

FIG. 2. *Disappearance of F pili after infection with Ff. A culture (uninfected or infected) of E. coli B/r Hfr, strain HB 42, which has only F pili and no common pili, was mixed with 100 UV-inactivated RNA phage per bacterium at 0 C to stain the F pili. Samples were prepared for microscopy by the agar filtration method, fixed with osmium tetroxide, and shadowed with platinum-palladium. (a) Uninfected HB 42 was grown to 2 × 10^8 cells/ml, sheared, washed, and grown for 30 min to regenerate F pili. Most of the bacteria showed attached F pili. (b) A parallel culture of HB 42 was infected with fd·B, grown to 2 × 10^8 cells/ml, sheared, washed, and grown for 30 min. No F pili stained by RNA phage were observed, but large amounts of fd were seen near unlysed cells. The bar represents 100 nm. (Malone and Marvin, unpublished data).*

GENETICS

Introduction

Since the genome of FV carries only a small amount of genetic information, it is feasible to define the functions of all virus genes. Ff strains have about 6,600 nucleotides which can code for 2,200 amino acids, or about 10 medium-sized proteins. Thus far, eight genes have been identified in Ff. Possibly there are only eight, although there may be still undiscovered genes. The additional 3,300 nucleotides found in If may code for more proteins in these viruses (perhaps because more virus functions are needed in the different hosts) or may simply carry redundant information or nonsense.

Most of our knowledge of the genetics of Ff comes from the work on M-13 (142, 143, 145, 146), although mutants of f1 (157, 165, 167, 168), fd (91, 166), HR (94, 95), and δA (194, 195, 198) have also been studied. The f1, fd, and HR mutants have been assigned to the corresponding M-13 genes by complementation with the M-13 tester mutants (145); thus, the genetics of these four viruses can be discussed together.

Mutagenesis

Chemical mutagenesis of a single-stranded DNA virus in vitro presumably takes place by transitions not transversions. Hydroxylamine is the most specific agent, inducing only C → T transitions (43, 134, 186, 187), and is also the most effective mutagen for Ff (145). Nitrous acid, ethyl methanesulfonate, and ultraviolet (UV) irradiation have also been used for Ff mutagenesis (47, 91, 145).

Mutagenesis of Ff can also conveniently be carried out in vivo on infected bacteria, since infected cultures continue to produce virus without lysis. The mutagens 8-azaguanine, 5-bromodeoxyuridine, proflavine, ethidium bromide, 2,7-diaminofluorene, 2-aminofluorene, 2-acetaminofluorene, and 2-(*N*-acetyl-hydroxylamino)-fluorene have all been used for in vivo mutagenesis (28,82–84, 145). Proflavine and ethidium bromide are mutagenic only in light, suggesting involvement of a photoreaction (83).

Plaque-Type Mutants

Plaques of FV in the double-agar-layer method are relatively small (132, 135, 162; Fig. 3). Plaque size can be increased by reducing top agar concentration to 0.35%; this low top-agar concentration is especially advisable for plating If (129). Virus mixed with indicator and plated immediately gives variable plaque size, probably because adsorption of virus to cells is slow. Thus plaque size is unsatisfactory as a genetic marker. More uniform plaque size can be obtained if preinfected bacteria are plated.

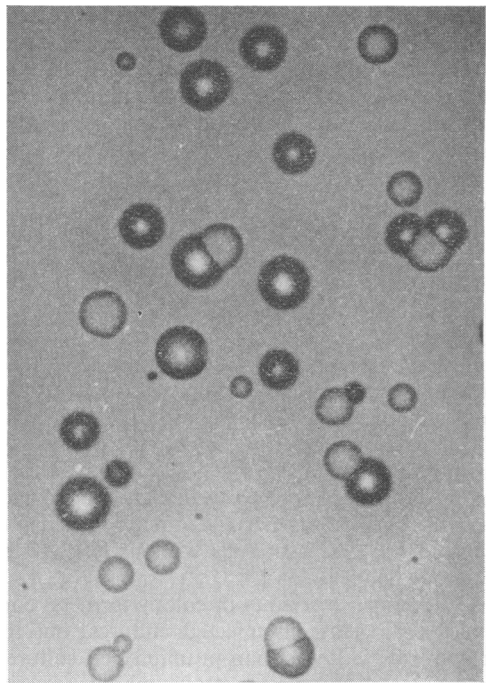

FIG. 3. *Plaque types of Ff. A sample of fd was plated on Giemsa plates, and the plates were photographed with transmitted light. Wild-type plaques have a characteristic centered appearance, because of regrowth of cells in the center of the plaque. Turbid plaques are uniform and are not much clearer than the bacterial lawn. The largest plaques are about 1.5 mm in diameter.*

Plaques of Ff can be more easily distinguished if the bottom agar contains 0.8% Giemsa stain solution (Fisher no. So-G-28). Plaques are then dark blue on a pinkish background. Infected colonies can also be distinguished from uninfected colonies on Giemsa plates: infected colonies are blue and uninfected colonies are slightly pinkish (B. Hohn, *unpublished data*). Large distinct plaques of fd are obtained on a special eosin-methylene blue bottom agar (18). The resolution of M-13 plaques is improved by using a tetrazolium staining technique (141). Plaques of Pf are revealed more clearly if bottom agar contains 0.006% methylene blue (132).

The optimum temperature range for forming Ff plaques is 37 to 43 C. Fairly good plating efficiency is observed at temperatures as low as 31 C, but poor plating efficiency is found below that temperature. Thus, the permissive temperature chosen for incubating plates of temperature-sensitive mutants is 33 C (145).

Wild-type fd plaques often have a ring or "centered" appearance as a result of regrowth of bacteria in the middle of the plaque (Fig. 3). Wild-type M-13 is clearer than fd.

When plates showing Ff plaques are reincubated below 32 C, the plaque may become the focus of further cell growth. Cells infected with strains carrying a defective but functional gene 2 do not show this effect (95). The effect may be related to abortive transformation in oncogenic viruses (95), or it may be a metabolic or other effect (45), perhaps related to the formation of papillae on bacterial colonies.

A range of mutants in plaque turbidity has been observed (95, 132, 162, 204; Fig. 3). The correlation of plaque turbidity with growth rate in infected cultures (*see* "Ecology") suggests that plaques are formed by a retardation of the growth rate of infected bacteria on the bacterial lawn. There are some indications that plaque turbidity is affected by mutation in gene 2, a gene controlling viral DNA replication. Gene 2 mutants of M-13 consistently show turbid plaques under permissive conditions (145). The vague plaque (VP) mutant of HR, which gives a very turbid plaque, has a defective gene 2 (95). Mutants of fd which show unstable plaque types and sectoring of plaques have been reported. Since the instability remains even after reisolation from single plaques, the mutants were interpreted as "mutator mutants" in the gene controlling DNA replication in the virus (84).

Conditional Lethal Mutants

Conditional lethal mutants, either temperature-sensitive or suppressor-sensitive, are most useful for physiological studies. All suppressor-sensitive mutants of Ff studied thus far have been *amber* mutants, i.e., those suppressible by UAG suppressors.

No good selection method for conditional lethal mutants of Ff has been found. Some preselection for temperature-sensitive mutants was obtained by incubating plates of mutagenized virus for 5 hr at 33 C and then shifting to 42 C overnight; large plaques were wild-type, whereas about 1% of the small plaques were mutants (145). A preselection for *amber* mutants of fd was based on the fact that turbid-plaque virus gave clear-plaque *amber* mutants on the permissive strain KB3 (91); this technique tends to give mutants in gene 4.

A total of 154 conditional lethal mutants of M-13 were assigned to six complementation groups (145, 146). The standard complementation test involves double infection of the nonpermissive host with two different mutants and then plating under nonpermissive conditions. Progeny virus is formed by the host if the two mutants complement each other. Plaques are formed under nonpermissive conditions only if both types of mutant virus are released from the double-infected cell. Thus, plaques are observed in this test only if complementation is symmetrical. M-13 mutants all showed symmetrical complementation (145). Complementation with the VP gene 2 mutant of HR may be asymmetrical (95).

Strong complementation was found between representatives of most groups. However, complementation within the set 1, 3, 6 was weak (with the exception of the one mutant, *am* 3-H5). This weak complementation may arise from the fact that groups 1, 3, and 6 form an operon. Under this assumption, the order of the

TABLE 2. *Functions of Ff virus genes*[a]

Gene	Function	Temp shift		Behavior of *amber* in a nonpermissive host						
		Time of block (shift down)	Continued need (shift up)	RF formed	RF synthesis	VS synthesis	RF density shift	Excess membrane	Serum blocking power	Host killed
Wild-type				+	+	+	+	−	+	−
1	Unknown	Late	Yes	+	+	+	+	+	−	+
2	RF replication	Early	No	+	−	−	−	−	−	−
3	A protein	Late	Yes	+	+	+	+	+	+	+
4	Unknown	Late	Yes	+	+	+	+	+	−	+
5	VS replication	Late	Yes	+	+	−	+	+	−	+
6	Unknown			+	+	+	+	+	+	+
7	Unknown									
8	B protein			+	+	+			−	

[a] References 81, 95, 143–146, 157, 165, 166; B. Y. Tseng (*personal communication*).

genes would be proximal-3-6-1-distal, using the rule that a polar mutant will complement weakly with any mutant in a group distal to it (146).

Since the original study, two additional M-13 complementation groups have been found (144). We will use the word "gene" (rather than "cistron") to refer to the subdivision of the genome defined by complementation tests (78). The genes and their known functions are listed in Table 2. In the next section, we will discuss in more detail some special points about the various genes.

The Genes

Gene 1. Production of double-stranded, replicative-form (RF) DNA and viral single-stranded (VS) DNA is normal in gene 1 mutants under nonpermissive conditions. However, serum-blocking power and viruslike particles are not observed (145, 166).

Gene 2. When this gene is defective, infecting VS is converted to RF, but no further DNA replication is observed (143). Polyacrylamide gel electropherograms of viral proteins from nonpermissive cells infected with *amber* 2 mutants show the same peaks that are observed in cells infected with wild-type virus. An additional peak, which may be a fragment of the gene 2 product, is also observed in the electropherogram. The fact that normal amounts of protein are made, although only the first RF is formed, suggests that only one RF molecule is normally involved in transcription (81).

In general, nonpermissive hosts infected with *amber* mutants show an accumulation of excess membrane in the cell, as observed in electron micrographs of thin sections of cells. Mutants in gene 2, alone among the genes 1 through 6 that were tested, did not show this effect (165).

In most cases, host cells infected under nonpermissive conditions with *amber* or temperature-sensitive Ff mutants no longer grow or form colonies (*see* "Killing of Host Cells"). Gene 2 mutants do not show this effect (146).

Growth of infected cells is inhibited by antiserum against Ff (*see* "Antiserum Effect"). Permissive bacteria infected with some *amber* 2 mutants or a gene 2 leaky defective mutant do not show this antiserum sensitivity (94, 95). (Presumably both of these situations give an altered but still functional protein.) Although the antiserum effect is not understood, these experiments may be interpreted to indicate an effect of gene 2 on the cell envelope.

Taken together, these observations suggest that the gene 2 product affects not only RF replication but also the membrane of infected cells. RF replication in ϕX174 is thought to take place at a membrane site and to involve a membrane-associated product (176, 178). Perhaps gene 2 in Ff is involved in formation of such a site.

During the course of normal infection, the infecting parental DNA becomes incorporated into the bacterial DNA: the density of the infecting parental label shifts from 1.701 to 1.710 g/cm^3. This effect is also observed under nonpermissive conditions for strains carrying mutations in genes 1, 3, 4, 5, or 6, but not mutations in gene 2 (B. Y. Tseng, *personal communication*).

Gene 3. This gene codes for a minor coat protein component, the A protein, which is involved in adsorption of virus to the host and in holding together the virus structure. Gene 3 mutants grown in nonpermissive conditions produce noninfectious particles containing infectious DNA. Some mutants produce polyvirus, which consists of long strings of viruslike particles attached end-to-end with no apparent limit on length, as if the machinery for ending the virus were defective (81, 144, 146, 157).

Ff and If adsorb to different sites on host bacteria. Thus, gene 3 is probably different for the two viruses.

Gene 4. Viruses carrying mutations in this gene are phenotypically similar to those in gene 1 (Table 2).

Gene. 5 This gene controls VS production. RF is replicated, but no VS is produced in nonpermissive hosts. The molecular weight of the gene 5 product is 10,000 to 12,000 atomic mass units (AMU), as calculated from its position in polyacrylamide gel electropherograms. In UV-irradiated cells, it is present in larger quantities than any other virus-specific protein: 10^3 to 10^4 molecules per cell at 35 min after infection. Thus, there are at least 10 molecules of the gene 5 product per RF during VS synthesis. The gene 5 product might have an enzymatic or only a structural role in promoting VS synthesis; in either case, the availability of the protein in relatively large quantities should simplify in vitro studies on VS synthesis (81, 143).

The formation of VS in ϕX174 requires the presence of viral coat proteins, but the gene 5 product of Ff is apparently not a coat protein.

Gene 6. Mutants with an altered gene 6 behave much like mutants with an altered gene 3. In nonpermissive hosts, they perform all virus functions up to and including the production of serum blocking power. After double infection of a nonpermissive host with an *amber* 6 and an *amber* mutant which is altered in another gene, synthesis of diploid (double-length) particles is stimulated. Maximum production of diploids is found

after double infection with a gene 6 mutant and a gene 3 mutant (146, 161).

Although there is no direct evidence on gene 6 function, these observations suggest that gene 6 is involved in ending the particle during morphopoiesis, perhaps at the end distal to the site of the gene 3 product.

Gene 7. Only one poorly characterized member of this class has been isolated; however, since it complements mutants in the other seven genes, it is put in a separate group (144).

Gene 8. This is the gene for the bulk coat protein (B protein). Mutants were isolated by growing mutagenized virus in permissive bacteria and then selecting for strains with altered density in a CsCl gradient. The mutants thus isolated have a reduced sensitivity to antiserum against M-13. The virions of some mutant strains show altered electrophoretic mobility. The B-protein peak in gel electropherograms is missing from extracts of nonpermissive bacteria infected with an *amber* 8 mutant. Synthesis of VS DNA is normal in nonpermissive bacteria infected with an *amber* 8 mutant, in contrast to the situation for ϕX174 coat mutants (81, 144).

Mutant fd strains have been isolated which show an altered mobility of the virus in carrier-free electrophoresis. These mutants are probably also gene 8 mutants (29, 30, 82).

Recombination

Attempts at genetic recombination between genes in Ff have been hampered by the presence of diploid particles (161, 168). These double-length aggregates are phenotypic recombinants, present to about 1% of the single-length particles. Although they may contain genetic information from two different parents (heterozygotes), this information is contained in two-single length DNA molecules. Thus, after replication, the heterozygotes give progeny of the two mutant types. This technical problem may be overcome in various ways (142). The molecular basis of recombination is not yet known, but analogy with ϕX174 suggests that an intermediate in recombination may be double-length rings (159).

The generation of transducing viruses by recombination between FV rings and another circular plasmid might be possible, although it has not been demonstrated. Since linear viruses are not limited to a certain genome content by the structure of the virion, they might be useful (or dangerous) in transferring genetic material from one organism to another.

Suppression of Amber Mutations

In single-stranded DNA, the amber triplet UAG may be formed by a single-base transition

TABLE 3. *Suppression of Ff amber mutations by various suppressors*[a]

fd Strain	Gene	Relative virus titer on various indicator strains					
		Su-1	*Su-2*	*Su-3*	*Su-4*	*Su-5*	*Sup⁻*
8	4	1	1	1	4×10^{-4}	4×10^{-4}	10^{-4}
9	4	1	1	3×10^{-5}	4×10^{-8}	6×10^{-8}	6×10^{-8}
10	4	1	1	10^{-5}	7×10^{-6}	9×10^{-6}	9×10^{-6}
11	4	1	1	1	4×10^{-4}	4×10^{-4}	10^{-4}
12	4	1	1	1	3×10^{-4}	3×10^{-4}	10^{-5}
13	4	1	1	0.5	5×10^{-6}	5×10^{-6}	5×10^{-6}
14	4	1	1	4×10^{-5}	10^{-6}	10^{-6}	10^{-6}
15	4	1	1	3×10^{-5}	10^{-6}	10^{-6}	10^{-6}
16	4	1	1	5×10^{-3}	7×10^{-4}	6×10^{-4}	6×10^{-4}
17	1	1	1	1	2×10^{-4}	2×10^{-4}	2×10^{-4}
18	2	1	1	1	2×10^{-5}	2×10^{-5}	2×10^{-5}
19	4	1	1	1	1	1	3×10^{-4}
20	4	1	1	1	10^{-5}	10^{-5}	10^{-5}
21	4	1	1	10^{-3}	10^{-6}	9×10^{-7}	3×10^{-6}
22	1	1	1	1	5×10^{-5}	7×10^{-5}	3×10^{-5}
23	4	1	1	4×10^{-3}	4×10^{-4}	4×10^{-4}	5×10^{-4}
24	5	1	1	1	0.3	6×10^{-4}	6×10^{-4}
26	4	1	1	2×10^{-3}	2×10^{-6}	10^{-6}	2×10^{-6}
28	4	1	1	7×10^{-3}	6×10^{-3}	4×10^{-3}	8×10^{-3}
29	4	1	1	3×10^{-3}	4×10^{-6}	4×10^{-6}	4×10^{-6}
30	1	1	1	1	2×10^{-4}	2×10^{-4}	2×10^{-4}

[a] Hydroxylamine was used for mutagenesis (186). Mutations were assigned to complementation groups with the M-13 tester mutants (145). Details of the suppressor strains of bacteria are given in reference 70.

only from CAG (codes for glutamine) or UGG (codes for tryptophan), since UAA is also a nonsense (*ochre*) codon. The three known suppressors (70) for the UAG mutation are *Su-1* (inserts serine), *Su-2* (inserts glutamine), and *Su-3* (inserts tyrosine). Since hydroxylamine mutagenesis of DNA promotes only C → T transitions, UAG must be derived from CAG, if the messenger RNA (mRNA) is homologous to the mutagenized DNA strand, and from UGG, if the mRNA is complementary to the mutagenized DNA strand.

Table 3 lists the pattern of suppression of a number of fd *amber* mutations. All mutations are suppressed by both *Su-1* and *Su-2*, but several are not suppressed by *Su-3*. The failure of *Su-3* to suppress hydroxylamine mutations which are suppressed by *Su-2* suggests that the UAG codon derives not from UGG (tryptophan) but from CAG (glutamine), since tyrosine (*Su-3*) should be a good analogue for tryptophan but a poor analogue for glutamine. From this argument, the mRNA has the same sequence as the mutagenized DNA strand. Viral strains which do not grow in *Su-3* can be induced to grow by the addition of 5-fluorouracil (Hohn and Marvin, *unpublished data*). This chemical is incorporated into mRNA in place of U, but is translated like C (43). Thus, the UAG codon is read as CAG, and glutamine is inserted in the protein.

The gene 3 mutant R4 shows wild-type resistance to alkali when suppressed by *Su-3*, but not when suppressed by *Su-1* or *Su-2*. Thus, the mutation probably arises from a substitution of UAG for UGG (tryptophan), since tyrosine (*Su-3*) is a better analogue than glutamine (*Su-2*) for tryptophan (157).

Most *amber* mutants of fd are poorly suppressed by *Su-4* and *Su-5*, the UAA (*ochre*) suppressors (Table 3). This effect may result from the low level of suppression by UAA suppressors (70), or it may result from a virus-induced alteration in the mechanism of translation (58), which hinders the reading of the UAG codon by the UUA anticodon.

STRUCTURE OF THE VIRION

DNA of the Virion

Infectious DNA can be isolated from FV by hot phenol or by phenol plus sodium dodecyl sulfate (SDS); further purification can be obtained by precipitation with alcohol (19, 87, 127, 135, 162). DNA can also be isolated by alkali treatment followed by banding in a CsCl density gradient (53, 157).

Purified FV DNA has a single-stranded conformation. (i) The nucleotide composition (Table 4) is not consistent with Watson-Crick pairing. (ii) The UV absorption at 260 nm [optical density $(OD)_{260}$] increases only gradually as a function of temperature, in contrast to the sharp melting points observed for double-stranded DNA (40, 87, 136, 162, 183). (iii) The OD_{260} and the sedimentation coefficient are strongly affected by ionic strength or divalent cations, indicating a more flexible molecule than double-stranded DNA. (iv) The inactivation of infectivity by UV is more rapid than expected for double-stranded DNA of the same size (47, 136, 150).

Purified FV DNA has a ring topology, with no

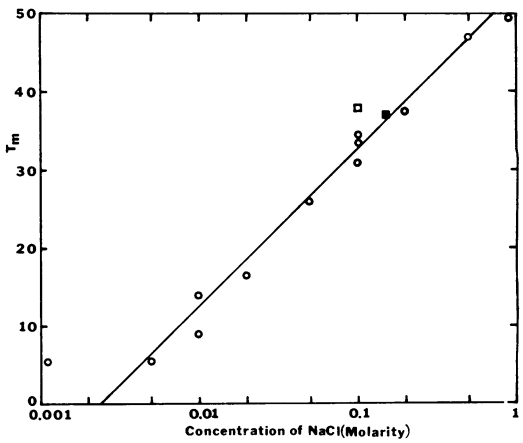

FIG. 4. *Variation of T_m of Ff DNA with salt concentration. DNA was prepared from fd by hot-phenol extraction (127) and was dialyzed at a concentration of about 300 μg/ml against 0.2 M NaCl, 0.02 M tris (hydroxymethyl)aminomethane (Tris), 0.002 M ethylenediaminetetraacetic acid (EDTA) (pH 7.5) and then against 0.2 M NaCl, 0.02 M Tris (pH 7.5; dialysis tubing was presoaked in EDTA). For measurements in 0.01 M or 0.001 M NaCl, the DNA was dialyzed against 0.01 M NaCl, 0.001 M tris (pH 7.5). The DNA was shown by analytical ultracentrifugation to be homogeneous and free from small UV-absorbing material. The DNA was diluted 1:15 or 1:30 into the desired concentration of NaCl plus Tris-hydrochloride (pH 7.5), in which the molarity of Tris was one-tenth that of NaCl. Dissolved air was previously removed from the solvent by aspiration. The melting curves were measured from 10 to 85 C as described in reference 96, except that temperature was measured at each equilibrium point by inserting a thermometer in a cuvette filled with water in the temperature-controlled block. DNA in 1 M NaCl showed no change of OD_{260} until 20 C, and then the total rise in OD_{260} was from 0.8 to 1.0 relative OD. Therefore, for DNA in lower salt, which shows no plateau at low temperature, the T_m (in degrees centigrade) was taken to be the temperature at which 0.9 of the final OD is reached. Symbols: ○, our measurements; ■, data from reference 162; □, data from reference 87.*

TABLE 4. *Physical and chemical measurements on FV*

Virus	Virion						Viral DNA					References
	Length (nm)	Diameter (nm)	Weight (AMU)	Weight per cent DNA	$S_{20,w}$ (Svedbergs)	ρ CsCl (g/cm³)	Base composition (mole per cent) T	C	A	G	Weight (AMU)	
fd	760, 870	5, 5.6	11.3×10^6	12.2	40	1.29	34.1	21.7	24.4	19.9	1.7, 2.1, 2.3×10^6	53, 62, 87, 108, 123, 125, 126
f1	850	4.3–6.3		11.3								205
M-13	850, 900					1.29	35.8	19.8	23.3	21.1	2×10^6	129, 133, 154, 162
ZJ/2	830	6.8										22
Ec9	600, 900	5		14.8	39		34.3	20.7	24.4	20.6		40, 59, 129
AE2	800	5		14.0	43		32	21	26	21		140
HR	800	6				1.31						95
δA	830	6	17×10^6	10	46	1.30	33.1	20.0	25.7	21.2	1.7×10^6	136
Xf	858	6										113
If1	1,300	5.5	25×10^6	12	45						3×10^6	129; R. L Wiseman[a]
If2	1,300											129
Pf1	1,400			12								183
v6						1.32						135
fd Core							23	27	19.5	30.5	12,000	H. Schaller[a]

[a] In preparation.

noncovalent bonds in the ring. (i) Sedimentation and viscosity measurements show that the molecular weight of the DNA is unchanged by a single deoxyribonuclease hit, whereas the axial ratio, measured in solvents that extend the molecule, is increased as the result of a single hit (127). (ii) There are no free ends on infectious virus DNA that are accessible to exonuclease (127). (iii) Single-stranded DNA used as a template for DNA polymerase forms a double-stranded form which is seen to be a ring in electron micrographs (133).

There is no evidence for a non-DNA linker in the DNA ring (127, 133). However, about 1% of Ff DNA is much more resistant to exonuclease (after endonuclease hits have been introduced) than the bulk of the DNA. The nucleotide composition (Table 4) and the pyrimidine oligonucleotide pattern of this core of about 40 nucleotides are quite different from those of the bulk of the DNA. Thus, the core may be a specific region with unusual secondary structure which makes it resistant to exonuclease (H. Schaller, *in preparation*). The biological significance of this region is not known.

The mean weight of a salt-free nucleotide in Ff DNA is 308 AMU $\pm$ 1%, so that the weight of the sodium salt is 330 AMU. The mean number of nitrogen atoms per nucleotide is 3.57 $\pm$ 1%. These means are derived from the average of the base compositions shown in Table 4. To avoid the misunderstanding which may arise from the custom of reporting DNA molecular weights as the weight of the sodium salt, we will refer all values to the number of nucleotides per molecule. The values for the weight of Ff DNA which have been reported range between 1.7×10^6 and 2.3×10^6 AMU (Table 4), or 5,200 to 7,000 nucleotides.

Accurate measurements of contour lengths of RF molecules may be obtained with the Kleinschmidt spreading technique (106). However, the length of an RF molecule may change by 10% depending on the method of spreading (133), so this technique is of most value when used to compare two types of molecule. A length of 2.19 $\pm$ 0.07 μm was found for M-13 RF with conditions (spreading on water) which gave 1.88 $\pm$ 0.09 μm for ϕX174 RF: a ratio of 1.16 (154). In a similar study, fd RF was 2.15 μm $\pm$ 5% long where ϕX174 RF was 1.75 μm $\pm$ 5% long: a ratio of 1.23. Thus, Ff RF is 1.2 $\pm$ 5% times longer than ϕX174 RF when spread on water. The contour length of fd RF spread on 0.3 M ammonium acetate is 1.98 μm (H. Frank, L. A. Day, and B. Krafczyk, *in preparation*).

The weight of ϕX174 DNA by light scattering (173) was reported as $1.7 \pm 0.1 \times 10^6$ AMU or

5,500 nucleotides. But a value of 0.201 cm^3/g was used for the refractive increment, whereas other laboratories have used 0.188 cm^3/g (76). If the lower value for the refractive increment is correct, ϕX174 DNA would have 6,300 nucleotides. Assuming that the ratio of Ff to ϕX174 DNA weights is the same as the ratio of Ff to ϕX174 RF lengths, Ff DNA has 6,600 to 7,600 nucleotides. For several different types of duplex DNA, Kleinschmidt spreading on water gives 0.35 $\pm$ 0.02 nm/nucleotide pair, and spreading on ammonium acetate gives 0.3 $\pm$ 0.01 nm/nucleotide pair (S. Leighton and I. Rubenstein, *in preparation*). Thus, Ff RF with a length of 2.17 μm on water would have 6,200 nucleotide pairs, and Ff RF with a length of 1.98 μm on ammonium acetate would have 6,600 nucleotide pairs.

As a subjective best value for the size of Ff DNA, we chose 6,600 nucleotides $\pm$ 15%. That is 2.0×10^6 AMU for the weight of the polyanion, and 2.2×10^6 AMU for the weight of the sodium salt. It would be useful to have a careful study of the molecular weight of Ff DNA by light scattering, using a low-angle scattering photometer and a direct determination of the refractive increment of Ff DNA; by sedimentation equilibrium; and by end-group determination after introducing a single break.

DNA from If weighs about 1.5 times as much as DNA from Ff, as estimated by comparing the sedimentation velocity of the two types of DNA (Wiseman and Marvin, *in preparation*).

Single-stranded DNA in aqueous solution has a flexible conformation, with considerable base stacking under moderate conditions of temperature, *p*H, ionic strength, or divalent cation concentration. Changes in these parameters may change the molecular domain and the amount of base stacking. Molecular domain may be measured by sedimentation velocity, viscosity, or light scattering; base stacking is inversely related to OD_{260} (68, 101).

The sedimentation coefficient (S_{20}) of Ff DNA may be as low as 4*S* under conditions in which the molecule is fully extended, such as high *p*H or no salt, and may rise to 30*S* under conditions in which the molecule is compact, such as high ionic strength or high Mg^{++} (40, 126, 127, 143, 162).

The buoyant density of Ff viral DNA is 1.722 g/cm^3 in CsCl, relative to 1.710 g/cm^3 for *Escherichia coli* DNA, and 1.447 g/cm^3 in Cs_2SO_4, relative to 1.426 g/cm^3 for *E. coli* DNA (180).

The midpoint of the temperature-induced increase in OD_{260} (T_m) varies with salt concentration (Fig. 4). The slope of the line is 20 C per 10-fold change in salt concentration, about the same as for double-stranded DNA (44), although the line is shifted by about 50 C to lower temperatures.

The OD_{260} measured at 22 C increases by 20% as NaCl concentration is reduced from 1 to 0.01 M (87). The OD_{260} also increases by 15 to 20% as Mg^{++} concentration is lowered at constant temperature and ionic strength. This transition takes place over a 100-fold change in Mg^{++} concentration. The midpoint of the transition comes at 0.00001 M Mg^{++} for 20 C, 0.001 M NaCl, and at 0.01 M Mg^{++} for 37 C and 0.01 M NaCl (Marvin, *unpublished data*).

The T_m of fd DNA is 37 C when the NaCl concentration is 0.15 M (Fig. 4); for 0.1 M NaCl at 37 C the midpoint of the OD_{260} change with Mg^{++} is 0.01 M Mg^{++}. These observations suggest that, in vivo, fd VS DNA may be in a state of unstable equilibrium. This may be a consideration relevant to the mechanism of synthesis of VS DNA or to the mechanism of encoating DNA in protein.

The gross base composition of Ff is indistinguishable from that of ϕX174: both have about one-third thymine. The similar base composition is not due to similar nucleotide sequences but may be due to use of a common subset of codons (58).

Interaction of Ff DNA with poly U has been analyzed by density shift in a CsCl gradient. With 10:1::poly U:DNA concentration, there is an increase in density of 0.015 g/cm^3. This experiment indicates dA-rich clusters on the viral DNA. There is a smaller interaction with poly I, G, or poly C, and none is detected with poly A (169).

Actinomycin (116), daunomycin, and 2,8-diamino-10 methyl acridine (40) bind to purified Ff DNA; in all three cases, the absorption maximum at about 450 nm is shifted by about 5% to longer wavelength as a result of binding to DNA.

Investigation of host-controlled modification in filamentous virus suggests that FV DNA contains a small number of 6-methylaminopurine bases (10, 117). The number appears to be different for the various FV, since they differ from one another in their modification and restriction pattern (Table 10).

Proteins of the Virion

A protein. A minor protein of Ff was identified by labeling the virus with amino acids which are not present in the bulk protein and analyzing the protein by polyacrylamide gel electrophoresis. By analyzing protein from *amber* mutants, this protein was shown to be the gene 3 product. The protein weighs 70,000 AMU (from the gel position) and comprises 0.5% of the total viral proteins

(from the fraction of total leucine and valine which is found in the protein). Thus, there is probably only one A protein molecule per virion. Phenotypic mixing experiments also indicate only one A protein per virion. The gene 3 product has been implicated in adsorption of virus to host, and adsorption of virus takes place at the end of the virus. Thus, it seems probable that the A protein is the adsorption protein and is located at only one end of the virion (81, 144, 146, 157). This is consistent with studies on polarity of the virus, which suggest that only one end of the virion acts as an adsorption site (41, 66).

B protein. The bulk protein of Ff can be prepared by: (i) phenol separation of DNA, followed by methanol precipitation of the protein from the phenol phase (12, 107); (ii) denaturing the virus with formic acid, digesting the DNA with deoxyribonuclease, and dialyzing out the digestion products (12); (iii) denaturing the virus with alkali and then sedimenting the DNA (12*S*) away from the protein (4*S*; 157).

The purified bulk protein in aqueous solution tends to aggregate under a variety of conditions. This tendency may be traced to the hydrophobic nature of the protein and is common for many structural proteins. The protein is soluble in 0.2% SDS, 0.01 M NaOH, 8 M urea, or 6 M guanidine hydrochloride. Chemical modification of the protein, by succinylation, maleylation, or tetrafluorosuccinylation, gives a product which is convenient for enzymatic degradation (12, 107).

There is only one major component in the bulk protein. Protein isolated from the virion shows a single major band in gel electrophoresis. Arginine, histidine, and cysteine are all missing in the bulk amino acid analysis, which is unlikely for a mixture of proteins. The amino acid sequence determination indicates only a single major protein (12, 81).

The molecular weight of the B protein was originally thought to be about 10,000 AMU, both by N-terminal analysis and by ultracentrifuge measurements on the protein dissolved in 1% SDS (25, 28, 107). However, careful measurements of the number of C-terminal ends per weight protein gave 5,900 AMU for the weight of the polypeptide chain (12). The earlier chemical measurements were affected by incomplete recovery of the N-terminal group (12). The ultracentrifuge measurements of molecular weight may give a high value because of the difficulty of measuring protein molecular weights in SDS.

The sequence of fd B-protein (Table 5) has been determined (12, 26, 27, 28). From the known 49 amino acids, the weight of the protein is 5,169 AMU $\pm$ 0.5%. The number of nitrogen atoms per molecule is 56 $\pm$ 5%. The uncertainty arises from some doubt about amidation of carboxyl groups and acylation of amino groups.

The sequence can be divided into three parts: residues 1 to 20 define an acidic region, residues 21 to 38 are hydrophobic, and residues 39 to 49 define a basic region. The first region may be responsible for stabilizing the virus in aqueous media, the second region may be responsible for interaction between neighboring subunits, and the third region may be responsible for interaction with the phosphate groups of the DNA (12, 27). A lysine-rich fraction of histone has a similar distribution of residues: a concentration of lysine in the carboxyl-terminal half of the molecule and a concentration of acidic residues in the amino-terminal half of the molecule (37).

Various semi-empirical schemes have been proposed to predict the α-helix content of a protein from the amino acid sequence. (See reference 119 for comparison of some of these methods.) Rule I of reference 149, "if the sum of alanine plus leucine exceeds 20%, the protein is 100% helical," predicts 100% helix. Rule VI, a safe rule designed to predict with high probability only a proportion of the α-helix content, predicts a region of α-helix between residues 6 and 20 or a minimum of 30% α-helix. Other methods also predict α-helix between residues 6 and 20.

The amino acid compositions of the B proteins of fd, M-13, f1, and ZJ/2 are almost the same (Table 6). A similarity is also seen in the serological behavior of fd, M-13, and f1 (Table 7).

Other proteins. *Amber* mutants in gene 6, grown together with another *amber* in a nonpermissive host, form a relatively high proportion of double-length heterozygotes, which contain two separa-

TABLE 5. *Sequence of fd B-protein*[a]

1	5	10	15	20	25
H-Ala-Glu-Gly-Asp-Asp-Pro-Ala-Lys-Ala-Ala-Phe-Asp-Ser-Leu-Gln-Ala-Ser-Ala-Thr-Glu-Tyr-Ile-Gly-Tyr-Ala					
	30	35	40	45	49
Trp-Met-Val-Val-Val-Ile-Val-Gly-Ala-Thr-Ile-Gly-Ile-Lys-Leu-Phe-Lys-Lys-Phe-Thr-Ser-Lys-Ala-Ser-OH					

[a] From reference 12.

ble molecules of DNA packed end-to-end in one continuous coat (161). Thus, gene 6 mutants are defective in ending the particle, and it is conceivable that the gene 6 product is a minor protein component of the virion.

Gel electropherograms of purified virus coat proteins show a minor protein weighing about 2,000 AMU. The fraction of leucine plus valine in this protein indicates that there are about 40 molecules of this protein per virion. It is not yet clear whether this protein is actually a structural protein of the virus or only a contaminating polypeptide (81).

The Virion

Types of evidence. The determination of the molecular structure of FV is likely to require the synthesis of data from many different sources. The major evidence will come from X-ray diffraction and electron microscopy, but these data are inadequate for structure determination without further facts about the proteins and nucleic acids which make up the virus.

Various types of information are available. There are direct measurements of basic parameters such as length, chemical composition, etc. There are derived quantities, parameters of interest which may be obtained by calculation from the basic parameters. Often these two will overlap, since some parameters may be either measured directly or derived from other parameters. There is also indirect evidence, such as spectroscopic experiments, which are not direct measurements of molecular parameters but which can be interpreted to give helpful suggestions about structure. Finally, experiments on the controlled degradation and the reconstitution of the virus also give information on how the DNA and proteins are put together.

TABLE 6. *Comparison of the amino acid compositions (number per molecule) of the B proteins of some filamentous bacterial viruses*[a]

Amino acid	Virus			
	fd	M-13	f1	ZJ/2
Lysine	5	5	5	5
Aspartic acid	3	3	3	3
Threonine	3	3	3	2
Serine	4	4	4	4
Glutamic acid	3	3	3–4	3
Proline	1	1	1	1
Glycine	4	4	4	4
Alanine	9	9	8–9	10
Valine	4	4	4	4
Methionine	1	1	1	1
Isoleucine	4	4	4	4
Leucine	2	2	2	2
Tyrosine	2	2	2	2
Phenylalanine	3	3	3	3
Tryptophan	1	1	1	1
Total	49	49	48–49	49

[a] From reference 12.

Direct measurements. Some measurements on FV are given in Table 4. The parameters which we have chosen as the basic parameters (those which we believe to be most accurately known) are listed in Table 8 together with estimates of error.

The length of fd is 870 ± 10 nm as measured on specimens prepared by agar filtration, and 880 ± 10 nm as measured on specimens prepared by Kleinschmidt spreading with 0.3 M ammonium acetate as hypophase, by use of careful microscope calibration (53; Fig. 5). Numbers ranging as low as 600 nm have been reported (Table 4). There may be some change in length with conditions of observation, e.g., the repeat unit of the virus in a fiber shrinks by 2% on drying, but is is likely that some of the discrepancies are due to inadequately calibrated electron micrographs. In the absence of a careful comparison of different viruses by the same investigator, it seems best to

TABLE 7. *Serological relationships between FV*[a]

Serum	Virus									
	fd	f1	M-13	Ec9	HR	If1	If2	Pf1	Pf2	v6
fd	1		0.2	0.6		0.1	0.1	−	−	
M-13	0.25; *0.5*	*0.5*	1	0.25		<0.001	<0.001			
Ec9	1		0.4			0.1	0.1			
HR		+	+		+					
If1	<0.001		<0.001	<0.001		1	1			
If2	<0.001		<0.001	<0.001		1	1			
Pf1	−							+	+	−
Pf2									+	
v6									−	+

[a] Relative *K* values or qualitative relationships are given. Most values are from reference 129. The italicized values are from reference 162. The "+/−" values are from references 95, 132, and 135.

TABLE 8. *Basic parameters of Ff*

Property	Quantity[a]
A. Length of virus.....	870 nm ± 3%
B. Nucleotides per virus.	6,600 ± 15%
C. N/P.....	28.4 ± 2%
D. Weight of B protein..	5,169 AMU ± 0.5%
E. N/B protein.....	56 ± 5%
F. Weight of nucleotide (mean).....	308 AMU ± 1%
G. N/nucleotide (mean).	3.57 ± 1%
H. Meridional repeat....	1.61 nm ± 0.5%

[a] References and sources of estimated error are given in the text.

assume that all FV with reported lengths in the range 600 to 900 nm have in fact the same length. We will use the value 870 nm ± 3%. The length of If is about 1.5 times that of Ff (129). A plot of the length distribution of fd shows that a small percentage of the particles are 1.5 times the standard length (126).

The N/P molar ratio of fd is 28.4 ± 0.3 (53, 87; H. Schaller, *personal communication*). This value is the mean of three independent determinations.

Purified concentrated preparations of FV become turbid after standing for a few days because of the formation of birefringent paracrystals (87). Under suitable conditions, spherulites are formed (Fig. 6). These spherulites may settle out of the anisotropic phase, giving a final purification of the virus. In a typical experiment, fd at 15 mg/ml in 0.01 M Na_3PO_4 (*p*H 12) formed two phases with OD ratios of OD_{270}/OD_{245} of 1.32 for the top phase and 1.39 for the bottom phase. The latter value is typical for highly purified fd (87). The paracrystalline preparations of FV behave in other ways as typical cholesteric liquid crystals (J. Lapointe, *unpublished data*). For X-ray diffraction studies, paracrystalline preparations of FV may be oriented in fibers, with the long axes of the virions lying roughly parallel to the long axis of the fiber (123). From the X-ray diffraction patterns of such fibers (Fig. 7), it is easy to make precise measurements of the dimensions of the unit cell, the repeating structural unit in the crystal. The minimum width of the unit cell, obtained from fibers with various water contents, is 5.5 ± 0.2 nm (123, 130). Values ranging between 4.3 and 6.8 nm have been reported for the width of the virion measured from electron micrographs (Table 4). For crystallographic reasons, the number of virions passing through the unit cell must be integral; the comparison of X-ray with electron microscope evidence indicates that this number is one. This determination of the contents of the unit cell is confirmed by comparison of the weight of material in the unit cell with the mass per length of the virion (123, 130). Thus, the minimum center-to-center distance between virions in the fiber is 5.5 ± 0.2 nm.

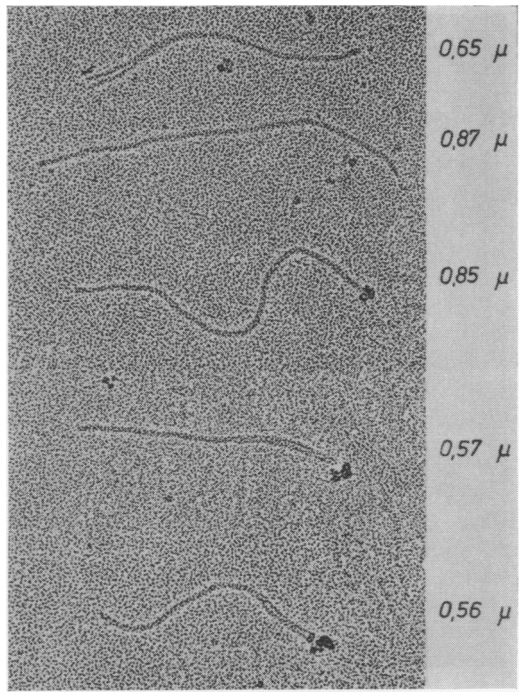

FIG. 5. *Electron micrographs of fd virions after partial alkaline denaturation. After a short incubation at pH 12.0 in 0.15 M NaCl, 0.02 M sodium phosphate, virus solutions were neutralized in Tris buffer (pH 7.2) alone (lower three) and in Tris buffer (pH 7.2) containing 4 M urea (upper two). They were prepared for microscopy by the agar filtration method followed by rotatory shadowing with platinum-palladium at an angle of 10°, and were examined in a Siemens-Elmiscope IA with magnification to the photographic plate of 20,000. The contour lengths given were obtained with a map measurer and 100,000-fold total magnification. Under the conditions used, single-stranded DNA appeared as dense irregular coils in the micrographs. The images shown were selected from different fields. The total incidence of particles which appeared to be split was about 2% in one experiment (Frank, Day, and Krafczyk, in preparation).*

The meridional reflections indicate that some structural subunit repeats regularly every 1.61 nm ± 0.5% along the length of the virion. This repeat distance shrinks by about 2% as the fibers are dried (123, 130). The layer line spacings at about 3.2 nm give the dimension of the unit cell along the virion.

Derived quantities. Some parameters of interest that can be derived from the direct measurements are shown in Table 9, together with the method of

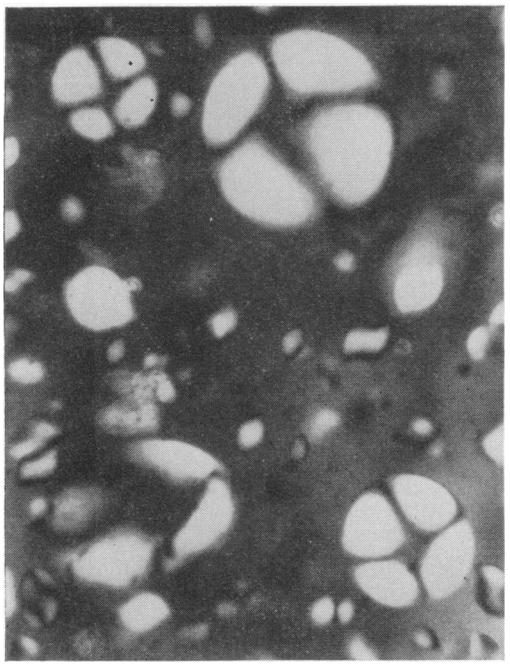

FIG. 6. *Spherulites of Ff. A drop of a 20 mg/ml solution of fd was placed on a glass slide, and a cover slip was placed 1 to 2 mm above the slide. As the solution dried, small birefringent tactoids appeared in the anisotropic phase and became gradually more spherical. These spherical structures show a black maltese cross under crossed polars. The measured retardation indicates that the higher index of refraction is tangent to the sphere. Since the intrinsic birefringence of fd is positive (123), it is probable that the virions lie with their long axes tangent to the sphere. The diameter of the largest spherulite is 0.2 mm (J. Lapointe, unpublished data).*

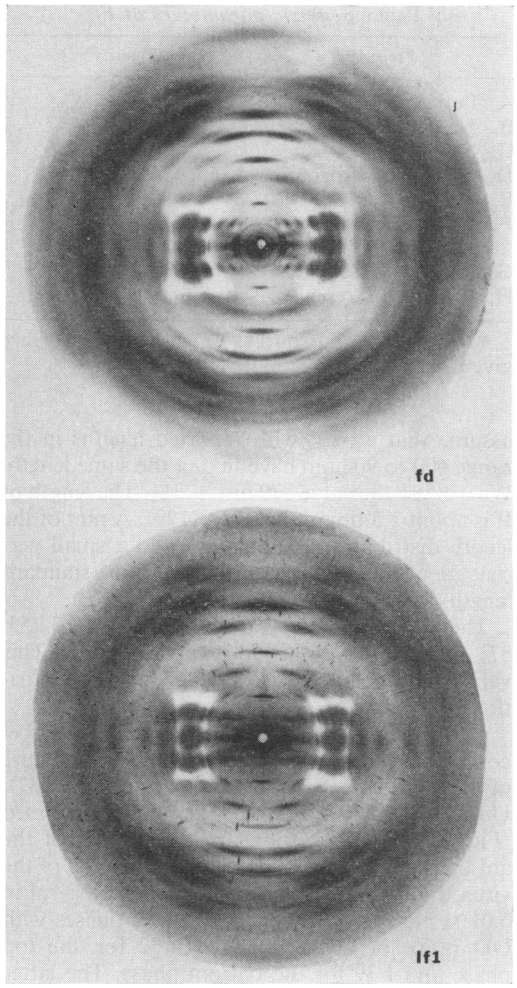

FIG. 7. *X-ray diffraction patterns of FV. The fiber axis (long axis of the virion) is vertical. Both patterns were obtained with standard methods (123) at 75% relative humidity. The strong 1.0-nm equatorial reflection has been shaded in the print in order to bring out more detail. The small sharp spots scattered over the If1 pattern are due to diffraction from salt (Wiseman and Marvin, in preparation).*

calculation. Some of these quantities have also been measured directly (Table 4). In the calculation of the weight per cent DNA and the weight of the virion, minor proteins have been neglected and the virion has been considered to be salt-free.

Indirect evidence. The strongest single feature on X-ray diffraction patterns of the virion (Fig. 7) is the intense region of diffraction at a spacing of 1.0 nm in the equatorial region (123). This feature indicates that much of the virion is composed of long, thin structures running roughly parallel to the virion axis, separated from each other by about 1.0 nm. Two types of diffraction patterns of protein fibers are known which show strong intensity at 1.0 nm on the equator: the α pattern and the β pattern (61). The β pattern, however, has an additional region of strong intensity at about 0.5 nm on the equator. Thus, the fd diffraction pattern most nearly resembles the α pattern. The α pattern has been interpreted in terms of the α-helix structure. This similarity of the X-ray diffraction pattern of fd to the α-helix diffraction pattern indicates that much of the fd protein is in the form of α helix.

Some electron micrographs of the virion show a thin, dark line running down the center of the virion in negatively stained preparations. This line has been interpreted in terms of a central DNA core in the virion (22), a hole down the center of the virion (23, 24, 41), or a groove between two parallel strands that might make up the virion

TABLE 9. *Derived parameters of Ff*

Property	Calculation[a]	Quantity
Weight of DNA (salt-free)	B × F	2.0×10^6 AMU ± 16%
Nucleotides per B protein	E/(C−G)	2.3 ± 8%
B proteins per virus	[B(C−G)]/E	2,900 ± 23%
Weight fraction DNA per virus	(E × F)/[E × F + D(C−G)]	0.12 ± 15%
Weight of virus (salt-free)	B[E × F + D(C−G)]/E	17×10^6 AMU ± 30%
Nucleotides per meridional repeat	(B × H)/A	12 ± 19%
B proteins per meridional repeat	[B × H(C−G)]/(A × E)	5.4 ± 27%

[a] Letters represented in Table 8.

(123). Electron micrographs showing splitting of virions (Fig. 5) support the last explanation.

Electron micrographs of sections of infected bacteria show many ringlike structures with dark central points. These have been interpreted as transverse sections of virions showing the central hole in the virion (23, 24). However, the diameter of these rings is more than 5.5 nm, so that they may not be single virions.

The isoelectric point of Ff is *p*H 4.1 (82).

A small precipitin reaction was observed between fd and antiserum against C-terminal serine, coupled to serum albumin. Inactivation of plaque-forming ability was observed with antiserum against proline and hydroxyproline, but not with antiserum against any other amino acid (1).

Measurements of absorption of polarized UV by oriented solutions of fd show differences in absorption depending on the orientation of the plane of the light relative to the orientation of the fd virions. One assumes that absorption near 260 nm is primarily due to the DNA of the virion, and that absorption at 280 nm is primarily due to the aromatic groups of the protein. The data then suggest that the planes of the DNA bases are tilted by about 20° (i.e., the angle between the long axis of the virion and the plane defined by the atoms in the bases is 70°). The data further suggest that the planes defined by the aromatic groups of the protein (especially tyrosine and tryptophan) are roughly parallel to the axis of the virion (17).

Measurements of optical rotatory dispersion on solutions of fd, after correction for the effect of DNA, indicate 75 to 90% α helix in the protein of the virion (12, 27, 52). Measurements of hypochromism in the far UV also indicate 80 to 90% α helix (53). These two types of measurements confirm the deduction, from X-ray data, of high α helix content.

The DNA in the virion is very hypochromic at 260 nm, suggesting a high degree of base stacking (53, 87). A large decrease in absorption centered at 290 nm after denaturation of the virion is ascribed to strong hyperchromicity of intact virus around 290 nm relative to separated protein and DNA. Such an effect could be due to a nonaqueous environment for tyrosine and tryptophan in the virion, or to head-to-tail alignment of their transition dipoles. Another source of this hyperchromicity could be n-π^* transitions in the DNA (53).

Study of solvent perturbation with D_2O-water suggests that tyrosine and tryptophan are more accessible to solvent molecules in the native virus than in the denatured virus (53). But native virus does not react with cyanuric fluoride, a tyrosine reagent, whereas the virus does react with this reagent after treatment with subtilisin (158). These two apparently conflicting results can be reconciled if the tyrosine is buried so as to be accessible to water but not to $C_3N_3F_3$ in the native virus, and if a slight conformational change resulting from subtilisin treatment permits cyanuric fluoride to react.

Controlled degradation. FV is resistant to many agents which inactivate other viruses, as indicated by the fact that in most preparations over half of the physical particles are infectious (87, 190, 205). Plaque-forming ability is resistant to deoxyribonuclease and ribonuclease and is moderately resistant to Pronase and trypsin; however, it is sensitive to the proteolytic enzymes Nagarse, ficin, subtilisin, and papain (132, 135, 158, 162, 168, 191).

Plaque-forming ability is not sensitive to freezing and thawing or to heating below about 80 C (22, 59, 60, 85, 87, 95, 102, 113, 129, 135). Thermodynamic calculations have been made from the heat inactivation data (60). All of the infectivity can be recovered after drying the virion under vacuum (60). The virion is relatively sensitive to shear or to ultrasonic treatment, because of its morphology (125, 135).

The virion is sensitive to chloroform (135, 200). Thus, it is preferable to use ether instead of chloroform to inactivate bacteria in a mixture of bacteria and FV. The virion is sensitive to SDS, although the degree of sensitivity depends on the strain; it is insensitive to sodium deoxycholate and Tween 80 below 1% detergent concentration (22, 200). It has the same sensitivity to nitrogen mustard as single-stranded spherical DNA and RNA phage (201).

The virion is stable between *p*H 2.5 and *p*H 10.5. Inactivation of the plaque-forming ability of Ff by alkali shows two different dependencies of rate constant on *p*H. Below *p*H 11.9, the rate of inactivation is much more dependent on *p*H than above this value. This behavior suggests a two-step titration of the protein: A protein is first titrated, converting the virion to an activated form in which the B protein can be titrated. An *amber* mutant which is altered in the A protein gene, grown in a suppressor strain which inserts serine or glutamine, is more sensitive to degradation by alkali than the wild type, as measured by the change of rate of inactivation with *p*H, by change in UV absorption, and by analysis in the ultracentrifuge (157).

Double-length heterozygotes are no more sensitive to subtilisin than normal length virions, which suggests that the site of subtilisin inactivation is at the end of the virion and not at the sides (168). After treatment with subtilisin, the virion has the same size and shape in low salt as the intact virion, as measured by sedimentation and viscosity. However, the subtilisin particle is sensitive to deoxyribonuclease and is degraded to DNA and protein by high salt. The experiments suggest that subtilisin attacks the A protein to give a "relaxed" particle which then disassembles in high salt (158).

These experiments on degradation by alkali and subtilisin and the electron micrographs of partially degraded virions (Fig. 5) indicate that the end of the virion is a particularly sensitive point on the virion.

Reconstitution. Purified viral protein and viral DNA can be reaggregated to irregular DNA-protein complexes. Reconstitution to infectious virions is possible at a low level (10^{-7} of the DNA molecules) if 3% "starter" protein is left on the DNA (108).

The degradation experiments suggest that damage to the end of the virion alters the whole virion. Since the structure of the whole virion depends on an intact A protein, the A protein may be a morphopoietic factor in the formation of the virion. It is not clear whether the A protein is related to the 3% starter protein needed for in vitro reconstitution.

Now that the A protein can be purified, a first step in reconstitution may be to attempt to combine the defective particles from gene 3 mutants, which lack the A protein, with purified A protein.

Model of the virion. The X-ray diffraction data on FV do not give details below about 0.5 nm. Probably the most fruitful approach to the determination of the molecular structure of the virion is that of model building. On the basis of all available data, a molecular model of the virion can be constructed and tested by comparing the observed diffraction pattern with that predicted by the model. The model is then revised (or discarded) according to the closeness of fit between observed and calculated patterns. This approach can in principle give a molecular model in which the position of each atom is known, since the known stereochemical structure of the subunits which make up the virion puts stringent limitations on possible models.

In discussing possible models, we assume that the DNA and the B protein are uniformly distributed along the shaft of the virion, with quasi-equivalent environments (42). For a linear virion, the assumption of equivalent environments implies a helical arrangement of subunits. Since the DNA is circular, two oppositely directed DNA strands pass along the length of the virion.

From Table 9, each 1.61-nm repeat unit along the length of the virion contains 12 nucleotides and 5.4 B protein molecules, or 2.3 nucleotides per protein. In each DNA strand there is one nucleotide every 0.26 nm. Since the planes of the bases can approach no closer than 0.34 nm, this nucleotide separation suggests that the planes of the bases are tilted away from the perpendicular to the helix axis. In the *A* structure of DNA, the nucleotides are 0.256 nm apart and the bases are tilted by 20°. A tilt of 20° was also deduced from the UV dichroism data on Ff. The protein seems to be largely in the α-helix conformation, with the axis of the α helix running roughly parallel to the axis of the virion.

Two general types of model have been considered for the structure of Ff. In one model, the virion is roughly a cylinder, with the DNA located in an axial hole down the middle of the cylinder (23, 130). In the other model, the viron consists of two parallel smaller cylinders, each consisting of a central single strand of DNA surrounded by protein (123). At present, the data are not sufficient to distinguish between the two models. Electron micrographs showing apparent hollow cylinders (24) support the first model, but electron micro-

graphs showing apparent splitting of two strands (123; Fig. 5) support the second model. Either model would be consistent with a tilted-base conformation for the DNA and an α-helix conformation for the protein. Until current X-ray diffraction experiments to distinguish between the two general types of model are completed, it does not seem useful to discuss the structure of the virion in more detail.

PHYSIOLOGY OF VIRUS INFECTION

Adsorption

Ff adsorb specifically to the tips of the F pili, and If adsorb specifically to the tips of the I pili (41, 128, 129; Fig. 8). There are only a few of these pili per bacterium (32, 41, 128), corresponding to the two to three adsorption sites found for Ff (95, 191). The adsorption sites apparently can be used only once, since infected cells cannot be superinfected, although they can be co-infected (85, 91, 95, 190). The small number of adsorption sites accounts for the observation that the adsorption rate constant is 100-fold lower than for T-even phages under similar conditions and also lower than for ϕX174 and RNA phage (85, 191).

The adsorption rate is maximal at 0.1 M NaCl and at similar ionic strengths of several other salts. Adsorption rate is the same between *p*H 5 and *p*H 8. There is no special requirement for divalent cations. Adsorption rate increases twofold between 0 and 40 C and then drops off sharply at higher temperatures. Thus, the rate behaves as if it were limited solely by diffusion and has no heat of activation (191).

Adsorption is not prevented by a temperature of 0 C, by 0.01 M KCN, or by 0.01 M sodium azide; thus, it requires no cell metabolism. Adsorption is irreversible; no conditions have been found under which the virus can be eluted from the cells after adsorption, without breaking off pili. Adsorption is prevented by 0.001 M Zn^{++} (191).

F pili are involved not only in adsorption of Ff but also in formation of mating pairs of bacteria and in adsorption of RNA phage. All three of these phenomena take place in the absence of cell metabolism. RNA phage adsorb to the sides of the F pili, whereas the F^- bacterium, like Ff, adsorbs to the tip of the pilus. Thus, formation of mating pairs and adsorption of Ff interfere with each other, whereas RNA phage adsorption is unaffected by Ff or by F^- bacteria at 0 C. F pili can be sheared off by blending and then are not regenerated at 0 C or in stationary-phase bacteria. Such blended cells do not adsorb Ff or RNA phage nor do they form mating pairs, but the free pili do adsorb Ff or RNA phage. Ff adsorbs to only one end of a free F pilus, and only one end of Ff adsorbs to this F-pilus site; adsorption is polar (32–34, 41, 50, 66, 98, 99, 138, 203).

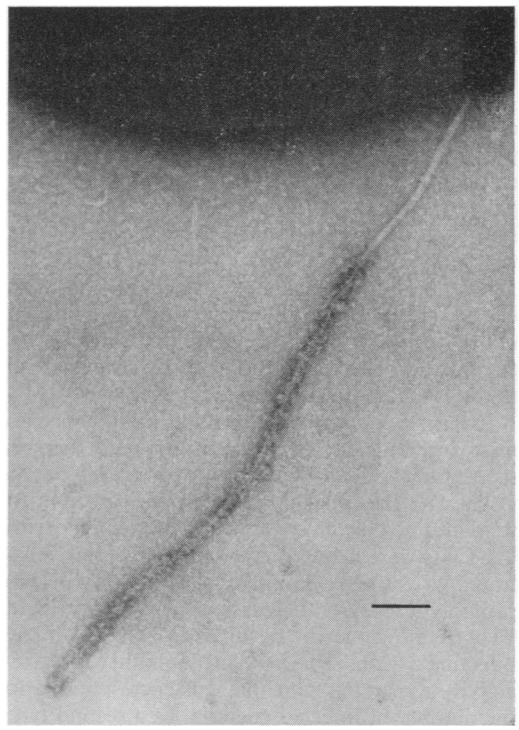

FIG. 8. *Attachment of Ff to pilus. M-13 adsorbed to the F-like pilus determined by colicin V2 was labeled with M-13 antibody (115). The virion appears fuzzy due to the antibody attached along the length. The bar represents 100 nm. (Electron micrograph kindly provided by A. M. Lawn and G. G. Meynell.)*

Bacterial mutants which adsorb Ff but not RNA phage have been isolated. Other mutants form no F pili (and therefore do not adsorb Ff or RNA phage) at 42 C but form pili at 34 C. These pili have normal resistance to heat, so that the lesion is presumably in pili assembly (172).

Penetration

Penetration of Ff is an energy-requiring step; it is prevented by 0.01 M KCN, by 0.01 M sodium azide, or by a temperature of 0 C. In this respect, it is similar to the formation of bacterial recombinants (50). There is a 20-fold increase in rate of penetration between 15 and 45 C. Unlike adsorption, penetration takes place efficiently at 45 C; thus, inhibition of adsorption at 45 C cannot be due to degradation of pili at the high temperature. Penetration is permitted but adsorption is prevented by 0.001 M Zn^{++}. There is no deoxyribo-

nuclease-sensitive step in Ff infection, although there is a ribonuclease-sensitive step in RNA phage infection (98, 191).

Competition experiments show that penetration of either Ff or RNA phage into male bacteria prevents the subsequent formation of bacterial mating pairs, but that neither virus affects mating pairs once formed. Penetration of RNA phage into bacteria prevents superinfection with Ff and vice-versa (33, 98, 99, 138).

Bacterial mutants have been isolated which can be infected by Ff and by the RNA phage $Q\beta$ but not by the RNA phage f2, unless very high multiplicities are used. Adsorption of f2 is normal, so that it is the penetration step that is blocked (171).

The most significant experiments on the mechanism of penetration of Ff are those which demonstrate that both the DNA and the protein of the virus enter the cell. In one type of experiment, fd was double-labeled with ^{3}H-thymine and with ^{35}S. Cells were infected and washed, and unpenetrated virus was removed by blending or with the proteolytic enzyme Nagarse. The ratio of ^{35}S to ^{3}H was the same in the infected cells as in the infecting virion. The absolute amount of radioactivity remaining with the cells corresponded to the number of infected bacteria. In a second type of experiment, fd was double-labeled with 5-bromouracil (BU), to give a density label in the DNA, and with a ^{14}C-amino acid mixture. Cells were infected and washed; the progeny virions released from the cells were analyzed by density gradient centrifugation 90 min after infection. Most of the radioactivity was present in light virions. Thus, the protein not only penetrates the cell but also is extruded as part of newly made virions. It is probable that the B protein is not degraded within the cell, since otherwise the amino acids would be largely retained in bacterial protein (189, 190).

The pilus extends from the cell envelope at a region where cell wall adheres to membrane (M. E. Bayer, *personal communication*).

One model which has been proposed for the function of F pili is the "F-pili conduction model." According to this model, the pilus is a hollow tube, with an outside diameter of 8.5 to 9.5 nm and an inside diameter of 1.5 to 2.5 nm; Ff DNA is injected at the outer end of the tube and transported towards the bacterium, RNA from RNA phage is injected into the side of the pilus and transported towards the bacterium, and DNA from the male bacterium during conjugation is transported away from the bacterium (32–34, 114). The main objection to this model is that it is difficult to conceive of a molecular mechanism by which a simple protein polymer like the F pili could actively transport three different kinds of nucleic acid (RNA; circular, single-stranded DNA; and linear, single-stranded DNA) in two different directions.

We favor the "F-pili retraction model" as an explanation for the function of F pili. According to this model, adsorption of RNA phage to the side, Ff to the tip, or an F^- cell to the tip of the pilus all trigger a retraction of the pilus. For instance, a slight change in the conformation of pilus protein might be transmitted from one subunit to the next down the pilus to the polymerizing mechanism at the base and here reverse the equilibrium monomer $\rightleftarrows$ polymer for pilus protein. By such a retraction, RNA phage would be pulled to the base of the pilus, where it injects its RNA; the F^- bacterium would be pulled up close to the male, where a classical conjugation bridge (4) is formed; and Ff would be pulled through the cell wall at the pilus site into the cell.

Some experiments support the retraction model over the conduction model. Early light microscope observations showed mating pairs to be apparently connected by invisible fibers. As the pairs were watched, the "mates would approach each other as though the fiber was being taken into or was becoming wrapped around one of the bacteria" (3). The retraction model predicts that RNA phage adsorption is not prevented by adsorption of Ff or F^- cells to F pili, whereas RNA phage adsorption is prevented by Ff penetration or formation of recombining pairs, because, in the latter situations, the pili have retracted and are no longer available as adsorption sites for RNA phage. This is, in fact, the situation which is observed, as discussed above.

The retraction model makes two further predictions which have been tested. No F pili should be seen in electron micrographs of cells infected with Ff, and the disappearance of F pili with time in a culture should parallel the appearance of bacteria infected with Ff. An experiment confirming the first prediction is illustrated in Fig. 2. Experiments to confirm the second prediction were carried out as follows. A culture of *E. coli* Hfr 3300 was sheared and washed to remove free F pili and then was allowed to regenerate F pili at 37 C in broth. The culture was infected with fd, using various multiplicities in different experiments. At suitable times after infection, samples were removed for measurement of the number of infected bacteria [with antiserum to remove free fd (190)] and the number of F pili [assayed by adsorption of radioactive RNA phage (33)]. The disappearance of RNA phage adsorption sites with time parallels the appearance of cells infected with Ff (A. F. Malone and D. A. Marvin, *unpublished data*). Many explanations for the disappearance of RNA phage adsorption sites after Ff infection

may be invented, but a simple explanation is that F pili retract into the cells.

The retraction model for F pili is reminiscent of some aspects of the behavior of nuclei and the mitotic spindle in eucaryotic organisms (13, 92, 97, 160).

DNA Replication

Introduction. DNA isolated from the Ff virion, like the DNA of ϕX174, is a single-stranded ring weighing about 2×10^6 AMU. Ff DNA goes through the same three stages (176) of replication as ϕX174 DNA: formation of a double-stranded RF, replication of the RF, and synthesis of VS. In so far as Ff DNA replication has been studied, the general pattern of DNA replication is the same for Ff as for ϕX174.

However, the replication of Ff DNA does differ in some details from that of ϕX174 DNA. Cells infected with Ff continue to grow and divide, unlike cells infected with ϕX174, so that Ff DNA replication has a steady-state stage in which viral and host DNA replicate together in log-phase-infected cells. There is a pool of VS found in cells infected with Ff, but this may reflect differences in the maturation of virus particles rather than significant differences in the mode of DNA replication.

Viral DNA replication is probably controlled by a few FV functions interacting with many host functions. Thus, an understanding of viral DNA replication should lead to increased understanding of host DNA replication.

In the following paragraphs, we discuss the known details of the stages of Ff DNA replication and mention some aspects of the mechanics, enzymology, and control of Ff DNA replication.

Stage I: formation of the unique RF. No more than about three viral DNA molecules enter the cell, even at high multiplicities, as though only one virus may enter through each of the adsorption sites (95, 190). The entering VS is converted to a double-stranded RF I (both strands being circular) by synthesis of a complementary strand (CS); labeled infecting VS is found in CsCl equilibrium sedimentation experiments at RF density (143; Fig. 12 and 13). This first RF to be formed from the entering VS we call the unique RF (URF). There may be more than one URF per cell, but the number is small, corresponding to the small number of entering VS.

The formation of URF takes place in the presence of 100 μg of chloramphenicol (CM) per ml and in all known conditional lethal virus mutants under nonpermissive conditions (105, 143, 144, 182). Thus, its formation probably does not require the expression of any viral gene. URF is also formed in Rep$^-$ mutants of bacteria which do not replicate RF past this stage (57).

Single-stranded Ff DNA is infectious to spheroplasts prepared from either F$^+$ or F$^-$ cells, although about 10-fold less so than ϕX174 DNA (18, 19, 85, 127, 153). Thus, formation of RF from VS can take place in spheroplasts.

Stage II: replication of RF. Semiconservative replication of double-stranded RF DNA may be either exponential or linear; that is, all molecules might replicate semiconservatively or only one special molecule might replicate (Fig. 9). Exponential replication can be most easily distinguished from linear replication by measuring the rate of synthesis: in exponential replication, rate increases exponentially with time, whereas in

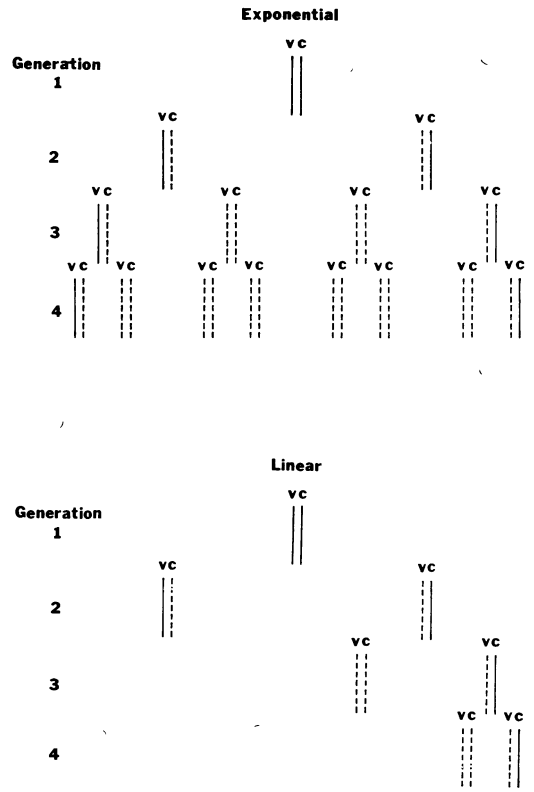

FIG. 9. *Two different models for DNA replication. RF molecules are here represented as linear, for ease of visualization, although they are in fact circular. The two strands of the parental RF, the infecting viral (V) strand and the first complementary (C) strand to be formed, are shown solid, and all strands formed later are dotted. In exponential replication, all RF, new and parental, replicate semiconservatively. In linear replication, as illustrated here, the first complementary strand is a stamping machine; only the RF which retains this CS can replicate.*

linear replication rate remains constant with time.

Figure 10 shows that the incorporation of pulses into DNA of log-phase-infected cells is exponential during the latent period and then becomes constant for about 1 hr. This effect is found for both thymine and thymidine pulses in a number of unrelated bacterial strains. The effect is not found with uridine pulses (Fig. 16). Therefore, it is probably not an artifact due to a change in cell permeability after infection (170), since we

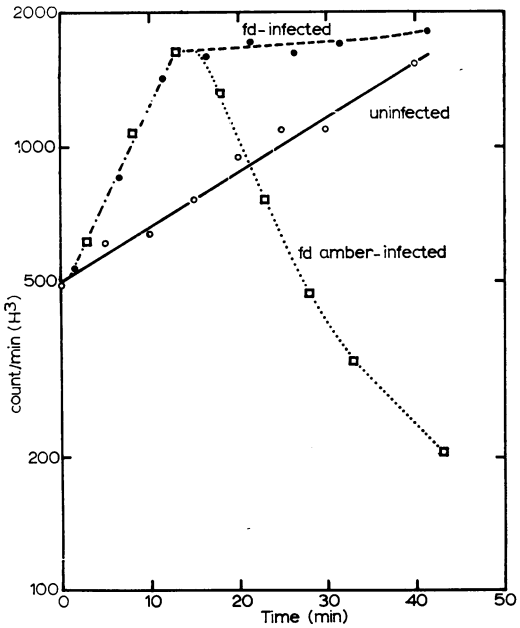

FIG. 10. *Rate of DNA synthesis in bacteria infected with Ff. A male derivative of E. coli 15 Thy⁻ His⁻ Ura⁻ Sup⁻ (74) was grown to 5 × 10⁷ bacteria/ml in 30 ml of glucose-Casamino Acids (TPG; 57) containing 20 μg of histidine per ml, 20 μg of uracil per ml, and 2 μg of thymine per ml, and was divided into three parts: uninfected, infected with 10¹⁰/ml of fd, and infected with 10¹⁰/ml of fd amber strain 9 (Table 3). At intervals, 0.5-ml portions of bacteria were added to tubes at 37 C containing 12.5 μc of ³H-thymine. After 60 sec, the incubation was stopped by the addition of 0.2 ml of iced 20% trichloroacetic acid, and 0.05 ml of a solution of 2 mg of denatured salmon sperm DNA per ml was added as carrier. After 30 min at 0 C, the precipitate was centrifuged, redissolved in 0.3 ml of 1 M NaOH containing 200 μg of cold thymine per ml, and reprecipitated with 0.15 ml of 70% trichloroacetic acid. After an additional 30 min at 0 C, the precipitate was rinsed onto B-6 membrane filters (Carl Schleicher & Schuell Co.) with 5 ml of 5% trichloroacetic acid, and the filters were dried, placed in toluene scintillator, and counted in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co.)*

expect that such a change would be the same for thymidine as for uridine. The rapid rise in rate during the first 10 to 15 min after infection is found in nonpermissive cells infected with mutants in genes 1, 3, 4, 5, or 6, but not with mutants in gene 2. (The decrease after 15 min is discussed in "Killing of Host Cells.") The nonpermissive cells infected with gene 2 mutants behave exactly like uninfected cells in these pulse experiments. Thus, the rapid rise in rate is a result of gene 2 function. Since gene 2 is required for RF replication (143), the experiments suggest that the difference between infected and uninfected cells results from RF synthesis. Since the difference increases exponentially, RF synthesis in stage II would be exponential, not linear.

Uninfected cells with a doubling time of 25 min have an average of 3.3 chromosomes per cell or about 10^7 base pairs (46). We assume that the incorporation of ^{3}H-thymine into trichloroacetic acid-precipitable material in 1-min pulses is proportional to the rate of DNA replication for both infected and uninfected cells in the type of experiment shown in Fig. 10. Integration of the curves in Fig. 10 from 0 to 10 min after infection gives a net synthesis of 6.4×10^6 bases per cell for the uninfected cells and 9.6×10^6 bases per cell for the infected cells. Net increase in the infected cell is 3.2×10^6 bases in the first 10-min period, which corresponds to 250 RF molecules.

Pulse experiments do not differentiate between turnover and net synthesis of DNA, if the turnover takes longer than the pulse. Experiments similar to that shown in Fig. 10 give curves similar to Fig. 10 even when the pulses are 5 min in length, suggesting that the rise is not due to turnover. The rapid rise in rate is also found after infection of RecB⁻ strains of bacteria, so that it is not a result of increased activity of the *recB* gene.

The technical problem arising from the presence of replicating bacterial DNA in infected cells may be eliminated by treating suitable cells with mitomycin C (MC), which stops bacterial DNA synthesis but permits RF synthesis. In a pulse experiment in cells treated with MC (MC cells), rate increased exponentially during the first 10 min after infection and then remained constant or decreased (166; Hohn and Marvin, *unpublished data*). This experiment suggests that initial replication of RF is exponential. Only about 40 RF molecules per cell were found at 30 min after infection in MC (143). One should be cautious about assuming that results obtained in MC cells apply quantitatively to normal cells, since virus production in MC cells may be less than in untreated cells (36, 95, 143).

Although the pulse experiments indicate that

replication of Ff DNA is exponential during the latent period, other experiments suggest that only one RF replicates. UV inactivation of the ability of infected cells to produce Ff follows single-hit kinetics, suggesting that one specific RF is required for production of Ff (95). Moreover, Ff is not produced if the CS of the first RF contains BU, even though the VS may contain BU and all later viral DNA may contain BU (Fig. 11). These experiments suggest that one RF (perhaps the RF which retains the CS of the URF) has a special role in Ff production. The evidence on ϕX174 indicates that ϕX174 RF is replicated by a "stamping machine" mechanism off a special, membrane-bound RF (176, 178). The RF which has a special role in Ff replication may be analogous to this special replicating RF in ϕX174.

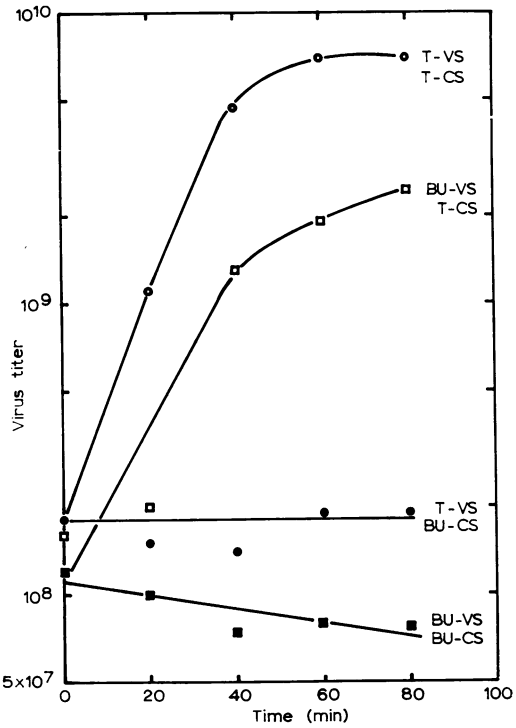

FIG. 11. *Failure of Ff to replicate if the parental RF is labeled with BU in the complementary strand. E. coli HfrH strain 165/1 Thy⁻ (from F. Bonhoeffer) was grown in 10 ml of TPG plus 20 μg of thymine per ml to 3.5 × 10^8 bacteria/ml, washed twice by centrifugation, and resuspended in two 5-ml portions of TPG without thymine. After 15 min of thymine starvation at 37 C, 20 μg of thymine per ml was added to one sample (T-CS), and 20 μg of BU per ml was added to the other (BU-CS). After 25 min of further growth, 60 μg of CM per ml was added to each tube, and incubation was continued for an additional 10 min. Each tube was then halved; one thymine tube and one BU tube were each infected (T-VS) with 10^9 fd/ml; the other thymine tube and the other BU tube were each infected (BU-VS) with 10^9 BU fd/ml (prepared according to reference 190). After 30 min, all four samples were washed twice and resuspended in 2.5 ml of TPG, to which 20 μg of BU per ml was added after 3 min. Increase in fd titer was measured for all four tubes.*

The available experiments leave us unclear about what really happens during stage II. On the one hand, there are suggestions (and the parallel with ϕX174) that only one RF replicates, which implies a linear increase in RF; on the other hand, there are suggestions that RF replication is exponential during the latent period. A scheme which could reconcile these conflicting results is presented in "Model of the Molecular Physiology of FV."

Stage III: synthesis of progeny viral strand. It is convenient to define the start of stage III as the point at about 10 min after infection when appreciable amounts of VS start to accumulate (91, 143).

Synthesis of progeny VS requires the gene 5 function (143) but may not require all of the bacterial functions which are needed for RF replication (147). The amount of infectious DNA in the cell rises exponentially to 25 VS equivalents per cell at 30 min after infection and 80 VS per cell at 40 min after infection, assuming that the contribution of RF to infective DNA is negligible (91, 112, 143, 153, 156). This is a doubling time of 6 min, about the same as the rate of virus release after the end of the latent period (Fig. 1). In MC cells, the pool is 15 VS molecules per cell at 30 min and remains small (36, 143). The exact size of the VS pool depends on the balance between VS synthesis and virus maturation and may be different for different conditions.

The rate of DNA synthesis, measured by incorporation of thymine during 1-min pulses, remains constant between the end of the latent period and about 1 hr after infection (Fig. 10). We define stage III to extend to the end of this period of linear DNA synthesis.

During stages II and III, the infecting parental VS disappears from RF. Some is released intact as progeny VS (7), and the remainder is incorporated into bacterial DNA. Labeled infecting VS is found in a CsCl equilibrium gradient at RF density early in infection but at bacterial DNA density later in infection. This effect can be observed for VS labeled with ^{32}P but no density label, because RF is 0.009 g/cm³ lighter than bacterial DNA (Fig. 12). The effect is also found for VS labeled with ^{32}P and BU; the ^{32}P appears at hybrid density early in infection and at bacterial density later in infection (Fig. 13). The fact that RF shifts to

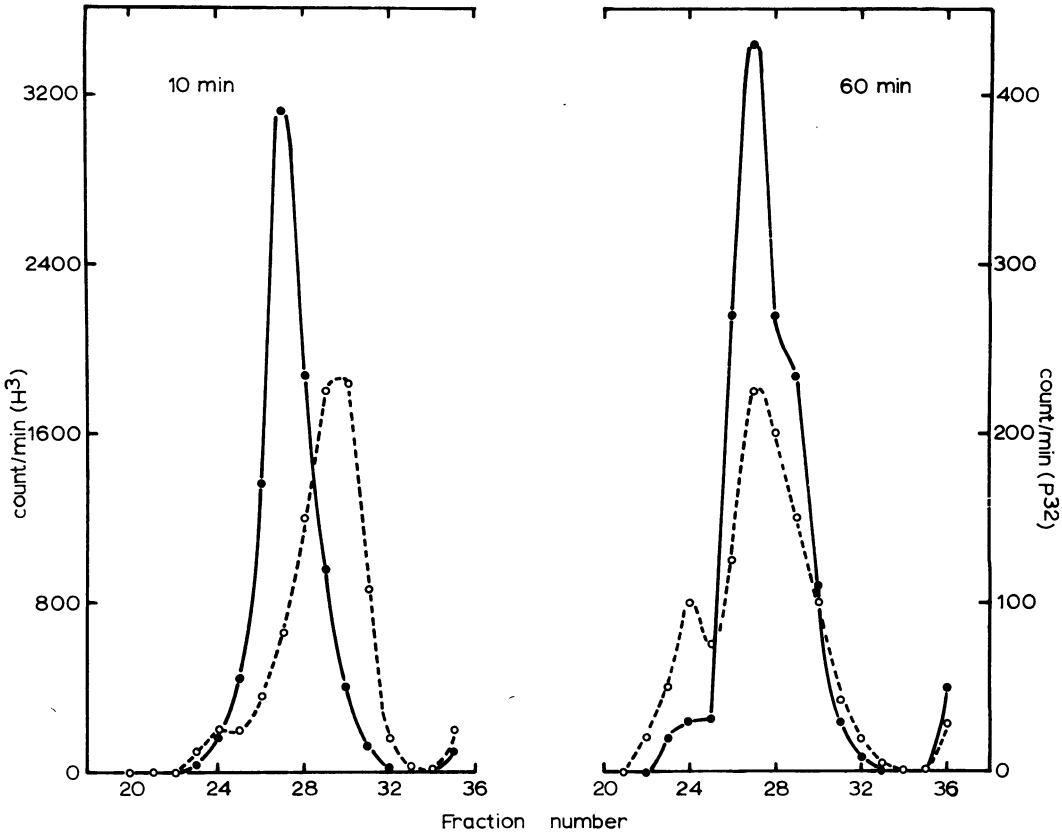

FIG. 12. *Shift of density of infecting Ff to bacterial density. E. coli CA 244 (from R. Epstein) was grown to 5 × 10⁸/ml in 4 ml of tryptone medium at 37 C, incubated with 0.2 μc of ³H-thymidine per ml for 10 min, and then infected with ³²P-fd at a multiplicity of 100. At 10 and 60 min after infection, 2-ml samples were washed, lysed with lysozyme-EDTA and freeze-thawing, and sheared to reduce viscosity. To 2.0 ml of sample, we added 2.65 g of CsCl, and the samples were centrifuged for 40 hr in the SW 39 rotor at 30,000 rev/min and 20 C. The tubes were punctured, and about 40 fractions were collected, precipitated, and counted in the scintillation counter. Solid lines are ³H, dashed are ³²P (from reference 91).*

heavier density in one case and lighter in the other rules out trivial explanations for the shift, such as attachment of mRNA, attachment of a protein, or change in DNA conformation. If the bacterial DNA isolated from a gradient of the type shown in Fig. 13 (60 min) is sheared, to give pieces weighing about 10^6 AMU, the counts remain at bacterial density, rather than skewing towards hybrid density. These and other experiments suggest that the incorporation of parental VS into bacterial DNA results not from insertion of an intact VS into the chromosome, but from breakdown of the parental VS, followed by reutilization of the components for new DNA synthesis (B. Y. Tseng, *personal communication*).

Stage IV: the carrier state. About 60 to 90 min after infection, an infected culture in log-phase growth enters a steady state, in which virus-specific DNA synthesis is not complicated by the transient effects which follow the shift from an uninfected to an infected culture. These bacteria may be thought of as being an altered strain which has the property of releasing infectious nucleoproteins. In this steady state, the 3×10^6 nucleotides of viral DNA in the cell (153) are distributed in the ratios RF I:RF II:VS::2:1:7 (152).

When bacteria infected with a strain of Ff carrying a temperature-sensitive mutation in gene 2 are shifted to nonpermissive temperature, the number of infected cells remains constant as the uninfected cells multiply (95), and the production of virus continues for 20 min and then stops (146). These experiments suggest that gene 2 must be expressed after each cell division in order to maintain the carrier state.

Under certain conditions, bacteria infected with

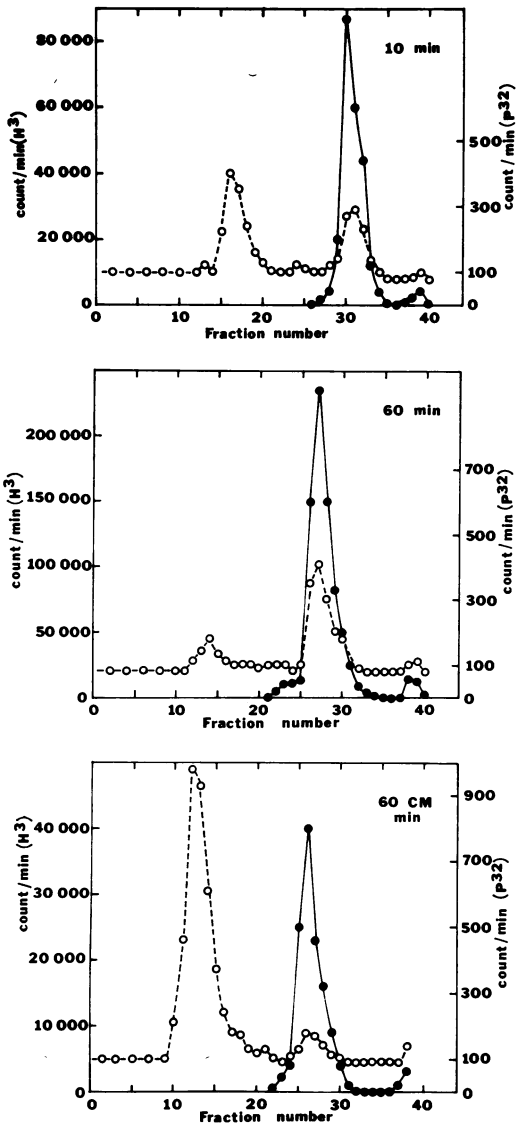

FIG. 13. *Shift of density of infecting heavy Ff to bacterial density. The preparation of fd containing BU instead of thymine is described in reference 190. E. coli Hfr 2340 was grown to 2 × 10⁸ bacteria/ml in tryptone medium, centrifuged, taken up at 37 C in 1 ml of tryptone medium plus 60 μg of CM per ml for 10 min, and then infected with fd labeled with BU and ³²P for 30 min. The bacteria were washed twice and resuspended in 10 ml of tryptone medium containing ³H-thymidine (0 min); 30 μg of CM per ml was added to half of this culture, and the two portions were incubated in parallel at 37 C. At 0 min there were 1.2 × 10⁸ colony formers and 1.7 × 10⁸ plaque formers/ml; after 60 min of growth there were 3.7 × 10⁸ colony formers/ml and 8.8 × 10¹⁰ plaque formers/ml; and after 60 min in CM there were 9.1 × 10⁷ colony formers/ml and 1.3 × 10⁷ plaque formers/ml. Samples were centrifuged in CsCl, collected, and counted as for Fig. 12. Refractive index of samples was measured in order to determine the CsCl density at which the DNA had banded. Samples at 10 min: 60% of the ³²P counts were at a hybrid density of 1.76 g/cm³, as expected for a double-stranded molecule with BU in the VS and thymine in the CS. Sample after 60 min growth: 20% of the ³²P was at hybrid density. Sample after 60 min in CM: 90% of the ³²P was at hybrid density. Solid lines are ³H; dashed lines are ³²P.*

FV release hardly any virus but retain the potential to make virus, even after many cell divisions. The viral factor in these cells has been called the metavirus. The metavirus state may be observed if gene 2 is defective but still functional; the character of the host strain may also play a role. The metavirus state may be cured with acridine orange (69, 75, 85, 88, 95, 132, 135, 181, 195, 197–199).

One explanation for the metavirus state is that RF replication does not function well enough to yield progeny virus; but one RF (the metavirus) still replicates once per bacterial generation, so that it is not lost by dilution. This explanation predicts some interaction between the regulation of RF replication and the regulation of the host cell DNA replication. Several models which are relevant to regulation of the replication of Ff DNA have been proposed (95, 124, 148, 198) and are discussed in more detail below.

When an infected cell divides, daughter cells may receive the capacity to produce virus (the metavirus) from within, or an uninfected daughter may segregate and then be reinfected from without. This fundamental question about the maintenance of the carrier state has not been satisfactorily answered. Attempts to prevent reinfection by adding antivirus serum are complicated by the fact that antiserum prevents division of infected cells (*see* "Antiserum Effect"). Infected cells do not yield uninfected daughters in micromanipulation experiments in which individual bacteria are separated and washed after cell division (88). But even in such experiments, the local concentration of virus near an infected cell is high, so that uninfected daughters could be reinfected at the moment of division.

Regulation. The maintenance of the carrier state may be compared with the maintenance of a plasmid or episome in the bacterium. The replication of the plasmid must be synchronized with cell division or else the plasmid either will be lost by dilution or will fill the cells. The frequency of bacterial cell division is controlled by the frequency of initiation of a round of bacterial chromosome replication (46, 122). Therefore, synchronization of plasmid replication with cell division becomes

a question of synchronization of plasmid replication with host DNA replication.

The replicon model for the control of DNA replication (discussed in references 51, 78, 122, and 148) assumes that each genetic element (replicon), such as a chromosome or plasmid, controls its own replication: there is a structural gene on the replicon which codes for an initiator of DNA replication. However, it is difficult, with this simple model, to explain the coordination between the replication of two different replicons in the same cell, such as a chromosome and a plasmid (95).

Two independent but related models for the regulation of DNA replication have been proposed to solve these difficulties. One general model (148) postulates an initiator of DNA replication *I*, which is produced constitutively so that the amount of *I* is proportional to cell size, and an inhibitor of initiation *H*, which is produced in a pulse, once per initiation, so that the amount of *H* depends on the frequency of initiation. Initiation takes place when the ratio of *I* to *H* is above a certain value. *I* may be common to both chromosome and plasmid, but each makes a unit amount of its specific *H* per replication.

In another specific model (124) of this type, *H* is a "site-territory" surrounding each membrane-bound site for DNA replication. Within this site-territory, new sites are not permitted (Fig. 14). As the cell membrane grows, the existing site-territories are diluted, and new sites are formed on the membrane; the surface area of the membrane is proportional to *I*. The chromosome and plasmid each makes its own type of site and therefore its own type of site-territory, but the amount of membrane available is the same for both.

Some authors have considered the possibility of a relationship between FV and extrachromosomal plasmids such as the F factor or colicin factors (2, 79, 80, 88). In both FV and these plasmids, the genetic material is a small circular DNA molecule, which may exist independently of the bacterial chromosome but which replicates in parallel with it. In both cases, the DNA codes for a structural protein that forms filaments and is involved in introducing the genome into bacteria which previously lacked it. The GC content of FV is 42%, and the RF weighs 4×10^6 to 6×10^6 AMU; an F-*lac* factor contains a unique region with about 44% GC which makes up 10% of the plasmid DNA, that is, about 6×10^6 AMU (65). The metavirus state of FV can be compared with the repressed state of transmissible plasmids (128).

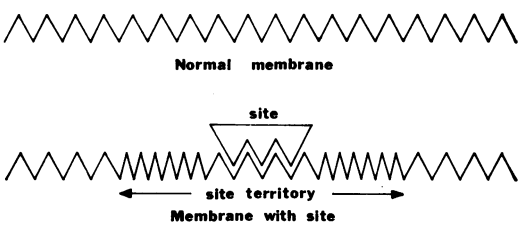

FIG. 14. *Illustration of the proposed change in membrane structure after binding of a site for DNA replication. Normal membrane is presumed to have a certain structure which is required for binding of the site to the membrane. Binding of the site to the membrane alters this membrane structure so as to prevent other sites from binding near the existing site.*

Mechanics and enzymology. The mechanical problems involved in semiconservative replication of a circular duplex DNA molecule have been discussed by many authors (38, 39, 51, 55, 73, 100, 111, 122, 139, 178, 188, 202). The replicating molecule has two discontinuities, the growth point and the origin (Fig. 15).

At the growth point of a replicating duplex, both the 3′ and the 5′ ends advance. On the other hand, single-stranded VS DNA requires the advancing of only a new 3′ end or a new 5′ end. The VS may be formed either by displacing an old VS with a newly formed VS or by synthesizing a new VS from a short template at the growth point (Fig. 15, bottom left).

The origin is not only the point at which replication starts but also the point to which the growth point returns after circumnavigating the DNA. Various possible structures for the origin are shown at the bottom right of Fig. 15. The rolling-circle model (73) has special appeal because one of the DNA strands may remain circular throughout many cycles of DNA replication.

There is some evidence bearing on the production of progeny VS. When ^{3}H-thymidine is added to infected bacteria in stage IV, label appears after 10 sec in RF and in a fast-sedimenting, heterogeneous, double-stranded component. Some of the single strands obtained by denaturing this heterogeneous component sediment faster than VS, as predicted by the rolling-circle model (73). Only the VS of the newly made RF II is nicked, and only the VS is chased from RF II into a single strand. Label appears in free VS DNA after 40 sec (152, 155).

Not much is known about the mechanics of RF replication. It is possible that instead of replicating semiconservatively, RF replicates by first forming VS and then converting VS to RF as in stage I.

Replication of Ff DNA may use the same machinery which is used for bacterial DNA repli-

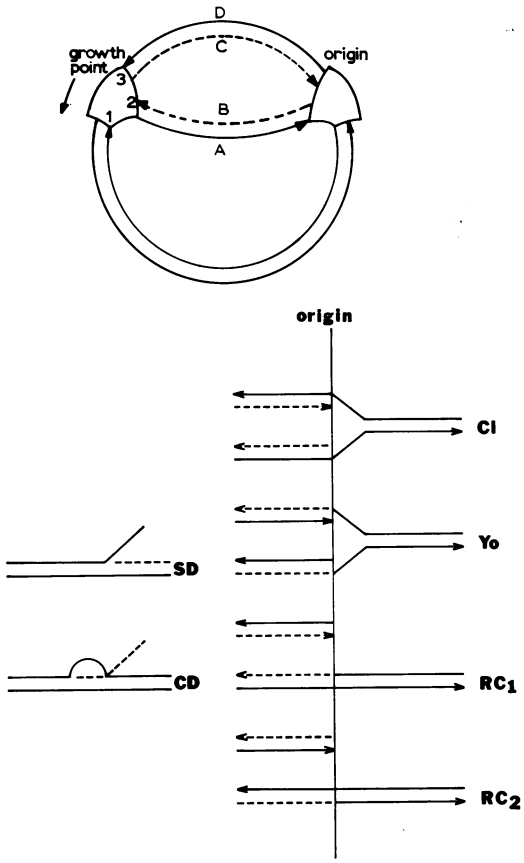

FIG. 15. *Mechanics of replicating a circular duplex DNA molecule. Solid lines represent DNA strands synthesized in the previous rounds of replication; dashed lines represent DNA strands synthesized since the last initiation of DNA replication at the origin. The arrows represent the 3' ends of the DNA strands. Top: the two discontinuities in the circle, the growth point and the origin. The growth point travels away from the origin, around the circle, and back to the origin, leaving two copies behind it. The growth point carries out three formally distinct functions: (1) separation of the two strands, (2) advancement of a new 3' end, and (3) advancement of a new 5' end. Bottom left: detail showing possible structures for the growth point during production of a single strand of DNA. SD is the semiconservative displacement model; it may be derived from the diagram at the top by removing either strand B or strand C from the diagram. CD is the conservative displacement model; the growth point leaves behind it a pre-existing duplex plus a newly made VS. Bottom right: detail showing possible structures for the origin. In each structure, part of the original RF is on the right, and the newly made double helices are on the left. CI is the classical model, which assumes that old strands separate while new strands are polymerized onto them. In the Yoshikawa (Yo) model (202), the old strands are broken at the origin, and the newly synthesized strands form extensions of the old strands. In both cases, if there is no break in any strand at the origin, a swivel must be postulated elsewhere in the circle. Otherwise it is necessary to introduce a break at the origin to facilitate uncoiling of the RF. This may occur in either strand of the RF. RC_1 is the rolling circle model with a free parental 5' end (73); RC_2 is the equivalent model with a free parental 3' end.*

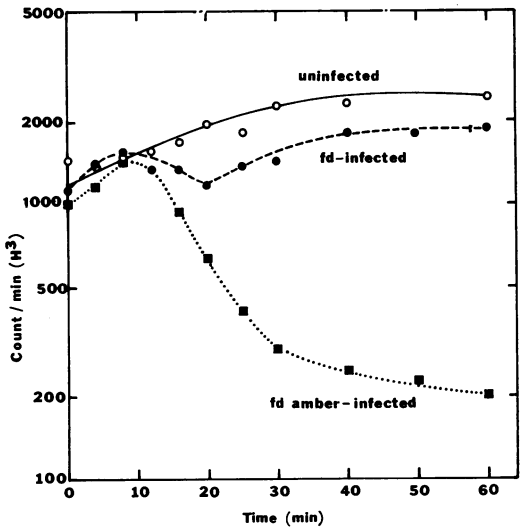

FIG. 16. *Rate of RNA synthesis in bacteria infected with Ff. A culture was grown and infected as in Fig. 10, except that the medium contained 20 μg of thymine per ml and there were 2 × 10⁷ bacteria/ml at 0 min. At intervals, 0.5-ml portions of bacteria were removed from the culture and added to tubes at 37 C containing 1 μc of ³H-uridine. After 45 sec, the incubation was stopped by the addition of 5 ml of 7% trichloroacetic acid; 0.5 ml of carrier bacteria (10⁹ bacteria/ml) was added, and the sample was filtered onto membrane filters, dried, and counted. In a parallel experiment, samples which were incubated overnight in 1 M NaOH showed no trichloroacetic acid-precipitable counts, indicating that no appreciable uridine had been incorporated into DNA.*

cation, or it may use machinery which is normally reserved for recombination or repair (93, 174).

The enzymes involved in replicating DNA in vivo are not known, but DNA replication may be carried out by DNA polymerase plus polynucleotide ligase (111). The synthesis of RF I from VS has been demonstrated in vitro for φX174 DNA (74). The formation of RF II from VS with DNA polymerase has also been demonstrated in vitro for Ff DNA (133), and presumably the formation of RF I with polynucleotide ligase is also possible. Linear pieces of DNA obtained by shearing Ff can be introduced into cells in the fragments of

Ff, but they are not replicated to a double-stranded form (67).

Purified RF I molecules show supertwists when examined in the electron microscope (154), suggesting a rotation between base pairs which is compensated for by twisting the chains around each other. Such a rotation could result if the DNA in RF I is in the *A* configuration in the cell but in the *B* configuration (with 10% more rotation per nucleotide) when spread for electron microscopy (48).

Transcription

The amount of ^{3}H-uridine incorporated into RNA during 45-sec pulses (Fig. 16) is slightly reduced by Ff infection. (The curve for infection with an *amber* mutant of Ff is discussed in "Killing of Host Cells.") Presumably, short pulses measure mostly incorporation into mRNA. Therefore, this experiment indicates that the total amount of mRNA synthesis is not appreciably altered by Ff infection.

Genetic experiments have been interpreted to indicate a polygenic mRNA for genes 3-6-1. If these genes are on one polygenic mRNA, then the other genes might be on a different mRNA. On the other hand, ϕX174 seems to be transcribed as one large mRNA (77). Messenger has the same base sequence as viral DNA (*see* "Genetics").

An artificial complex of Ff DNA and complementary RNA has been studied by X-ray diffraction (131). The structure remains in a configuration similar to the *A* configuration of DNA under conditions which would transform *A*-DNA to *B*-DNA. This fact may be relevant to models of transcription; perhaps DNA-dependent RNA polymerase acts only on DNA in the *A* configuration, and repressor acts by preventing the *B* to *A* transition (11).

Cells infected with strains of Ff carrying a mutation in gene 2, which make URF but do not replicate RF, show the same amount of viral proteins as cells infected with wild-type Ff (81). This experiment suggests that one RF may be sufficient for transcription.

Ff DNA is a good messenger in a cell-free system containing neomycin B (31). The free VS of Ff might act as a messenger in vivo. At least some expression takes place via mRNA, however, since *amber* mutants of Ff are reverted by 5-fluorouracil in the presence of thymine or thymidine but not in the presence of uracil (91; Hohn and Marvin, *unpublished data*).

Translation

The rate of protein synthesis in cells infected with Ff, as measured by incorporation of ^{3}H-amino acid mixture during 90-sec pulses, is reduced to 60 to 80% of the value in uninfected cells (Fig. 17). Similar experiments with bacteria growing in lactose instead of glycerol medium show a slightly different effect. The rate of protein synthesis parallels that of uninfected cells for the first 10 min, then remains constant for 30 min, and finally

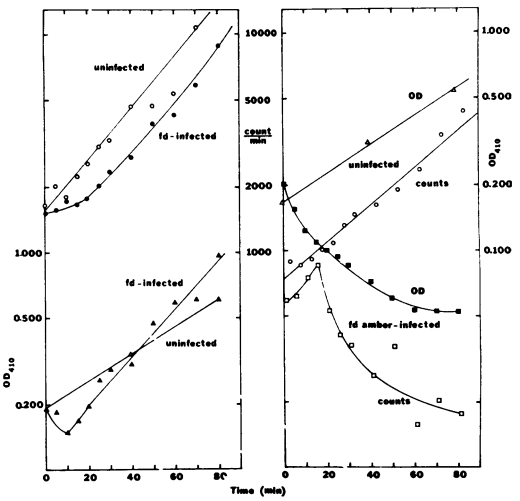

FIG. 17. *Rate of protein synthesis in bacteria infected with Ff. Bacteria were grown and infected as in Fig. 16, except that the medium contained 2 g of glycerol per liter instead of glucose. For measurement of the rate of protein synthesis, 0.5-ml portions of culture were added to tubes at 37 C containing 10 μc of ^{3}H reconstituted protein hydrolysate (Schwarz) and were incubated for 90 sec; the reaction was stopped with iced buffer containing 0.03 M KCN. A 0.2-ml sample of carrier bacteria was added; the bacteria were centrifuged at 0 C, taken up in 0.3 ml of 1 M NaOH, and incubated for 1 to 2 hr at 37 C. The samples were then precipitated with 0.2 ml of 70% trichloroacetic acid for 30 min at 0 C, washed on membrane filters, and counted in a toluene scintillator. For measurement of induced enzyme, 3 ml of bacteria was added to 0.3 ml of 0.01 M isopropyl-β-D-thiogalactopyranoside and was incubated for 9.5 min at 37 C. The induction was stopped by adding 2 ml of this mixture to 0.05 ml of toluene plus 0.05 ml of 0.1% SDS at 30 C. Incubation in toluene plus SDS continued for at least 60 min. Then 0.5 ml was removed and added to 3 ml of o-nitrophenyl-β-D-galactopyranoside (0.9 mg/ml) in 0.1 M Na_2HPO_4, 0.01 M $MgSO_4$ (pH 7.0). After 40 min of incubation, the reaction was stopped with 0.1 ml of 0.3 M KCN, and OD_{410} was measured. Left: incorporation of amino acids (top) into cells infected with fd (●) and into a parallel uninfected control (○). Induction of β-galactosidase (bottom) in infected (▲) and uninfected (△) cells. Right: incorporation of amino acids and induction of β-galactosidase in bacteria infected with fd amber 9 (Table 3) and into parallel uninfected controls.*

rises again parallel to the uninfected cells at 60 to 80% of the uninfected value. The rate of induction of β-galactosidase drops sharply during the first stages of infection and then remains at 60 to 80% of the uninfected value (Fig. 17). The reasons for these transient effects on protein synthesis after infection are not known.

Virus-induced changes in translation have been observed for several viruses (179). Indirect arguments indicate that such changes in translation are also induced by infection with FV (58). In normal cells, anticodons are thought to pair with more than one codon, according to the wobble hypothesis (49); a virus-induced structural limitation on wobble in infected cells may restrict the pairing of the anticodon to only one codon (58). The fact that *amber* mutants of Ff are poorly suppressed by *ochre* suppressors (Table 3) would be consistent with this idea.

Unusual structures which are apparently ordered polysomes have been observed in cells infected with Ff (165).

Morphopoiesis and Extrusion

FV is released from infected cells without lysis. There is no detectable pool of infectious intracellular virus. There is a pool of VS DNA in the cell but no detectable pool of B protein in the cytoplasm. Measurements of the delay before a pulse of amino acid label reappears in progeny virus indicate a maximum of 10 virus equivalents of B protein per cell (85, 88, 90, 95, 104, 105, 112, 162, 189, 196).

These observations indicate that the virus is assembled as it is extruded from the cell. The effect of antivirus serum on infected cells (*see* "Antiserum Effect") suggests the possibility that viral protein is stored within the membrane, where it is picked up by the DNA as the virus is extruded from the cell. Both parental protein, after being stripped off the virus upon infection, and progeny protein, waiting to be extruded, could have this location. A pool of 10 virus equivalents of protein would occupy less than 1% of the membrane surface, so that storage of viral protein in the membrane need not be directly related to the increase in membrane in infected cells (24, 165). Presumably, the protein pool would be concentrated in one region, near the site of virus release.

It is not known whether the A protein is attached first to the distal end or last to the proximal end of the extruding virus.

The fact that no pool of completed virus is found in the cells means that virus is continuously extruded from the cell and is not released in a burst during a certain stage in the bacterial life cycle.

The supernatant fluid of infected cultures contains free lipopolysaccharides, presumably released from the cell wall, which specifically inhibit methyl transferase. Purified inhibitor is masked by uninfected cells (64).

Several explanations may be suggested for the failure of in vitro virus assembly (108, 109). Maturation may be an active process which requires cell functions, so that it cannot be easily duplicated in vitro. This type of assembly has been called higher-order morphopoiesis (103). Another possibility is that it may be necessary to add viral components in a special sequence, as found for T-4 phage assembly. Lastly, the hydrophobic protein might take up the correct conformation only in a hydrophobic environment, such as the cell membrane. If this is the case, attempts at reconstitution in vitro from a lipid-water mixture might be fruitful.

INTERACTION BETWEEN VIRAL AND HOST FUNCTIONS

Restriction and Modification

Foreign DNA entering certain bacterial strains may be inactivated (restricted). A small fraction of the DNA which escapes this barrier is modified so that it is no longer subject to restriction. Progeny DNA is modified in the same way, so that it is no longer restricted when passed to a homologous host (6–10). The pattern of restriction and modification for filamentous virus is shown in Table 10.

The chemical basis of modification may be methylation of adenine. When fd is grown on *E. coli* strain B, to give virus with the modification pattern of strain B (fd·B), it plates with an efficiency of 1 on strain B. When fd is grown on strain 0, to give virus with the modification pattern of strain 0 (fd·0), it plates with an efficiency of 7×10^{-4} on strain B. Chemical analysis of the modified virus indicates two 6-methylaminopurines per fd·0 genome and four 6-methylaminopurines per fd·B genome. These experiments indicate that there are two B-specific sites on the fd DNA molecule. If both sites are methylated (fd·B), the restricting enzyme does not attack the DNA; if only one site is methylated, there is a 3×10^{-2} chance that the DNA will escape restriction; and, if neither site is methylated (fd·0), there is only a 7×10^{-4} chance that the DNA will escape restriction (10).

Infectious VS and RF molecules are equally well restricted in spheroplasts, but less so than in normal virus infection (18). Studies of restriction in vitro suggest that restriction takes place on RF but not on VS (117). Presumably, restriction takes place during stage I of RF replication.

TABLE 10. *Restriction of modified FV on various hosts*[a]

Virus	Host							
	0	K	B	$Kr_B^+m_B^+$	15	A	0(P1)	K(P1)
fd·0	1	1	7×10^{-4}	7×10^{-4}	1	1	0.3	0.3
fd·K	1	1	4×10^{-4}	7×10^{-4}	—	—	0.3	—
fd·B	1	1	1	1	1	1	0.3	0.3
fd*s*B-1°*s*B-*2*·0	1	1	3×10^{-2}	—	1	1	0.3	0.3
fd*s*B-1°*s*B-*2*°·0	1	1	1	—	1	1	0.3	0.3
fd·0, P1	1	1	7×10^{-4}	7×10^{-4}	1	1	1	1
f1·0	1	1	3×10^{-4}	6×10^{-4}	—	—	0.3	—
f1·K	—	1	3×10^{-4}	6×10^{-4}	—	—	0.3	—
f1·B	—	1	1	1	—	—	0.3	—
f1·0, P1	—	1	3×10^{-4}	6×10^{-4}	—	—	1	—
M-13·0	1	1	1.7×10^{-2}	2.4×10^{-2}	—	—	0.3	—
M-13·K	—	1	1.7×10^{-2}	2.4×10^{-4}	—	—	0.3	—
M-13·B	—	1	1	1	—	—	0.3	—
F12·K	—	1	1.6×10^{-2}	2.2×10^{-2}	—	—	1	—
F12·B	—	1	1	1	—	—	1	—
If1·0	1	1	4×10^{-3}	—	0.25	10^{-2}	2×10^{-2}	—
If1·B	1	1	1	—	—	—	—	—
If1·15	1	1	4×10^{-3}	—	1	—	—	—
If1·A	1	1	4×10^{-3}	—	—	1	—	—
If1·0, P1	1	1	4×10^{-3}	—	—	—	1	—
If2·0	1	1	4×10^{-2}	—	0.5	8×10^{-3}	0.4	—

[a] Modification of fd by K is represented by fd·K, etc. Numbers give relative virus titer observed on various hosts. For further details of nomenclature, see reference 10. Data are from references 7, 8, and 10, and from T. Bickle and W. Arber (*personal communication*). A dash indicates that no relevant data are available.

If fd DNA is applied to tobacco leaves and incubated, an increase in virus titer is observed. Apparently, the effect is not simply due to infection by DNA of bacteria associated with tobacco leaves. When fd·K DNA (essentially the same as fd·0) is applied to the leaves, the virus which is recovered plates as well on *E. coli* B as on *E. coli* K. Thus, growth of virus in tobacco seems to cause methylation of the sites which are recognized by the restricting enzyme of *E. coli* B (163, 164).

Killing of Host Cells

Normal infection by Ff does not lyse or otherwise kill the host. However, several conditions which hinder release of progeny virus from the host stop the growth of the host, after a 15-min lag, and destroy its colony-forming ability (91, 145, 146, 167; Fig. 18). This killing phenomenon is most conveniently studied by use of conditional lethal mutants of the virus. Strains of virus carrying mutations in genes 1, 3, 4, 5, or 6 kill the host under nonpermissive conditions, but gene 2 mutants do not kill. The effect is observed with both *amber* and temperature-sensitive mutants, so that it is not a polarity effect. Thus, if gene 2 functions, but any other gene is defective, the infected cell cannot form a colony (145).

The inactivation of colony-forming ability follows a single-hit curve (91). Thus, a single virion is sufficient to cause killing.

Double mutants in gene 2 and another gene do not kill, although double mutants with a functional gene 2 do kill (145). Killing is reversed by double infection with a killing *amber* and a wild-type virus (91). Thus, the killing property is recessive.

The crucial event leading to cell death appears to take place at the end of the latent period. Cell division and DNA, RNA, and protein synthesis are about the same as in normal infection until the end of the latent period and then drop sharply (Fig. 10, 16–18).

Rate of DNA synthesis, as measured by incorporation of 60-sec ^{3}H-thymine or ^{3}H-thymidine pulses into cell DNA, drops off sharply at the end

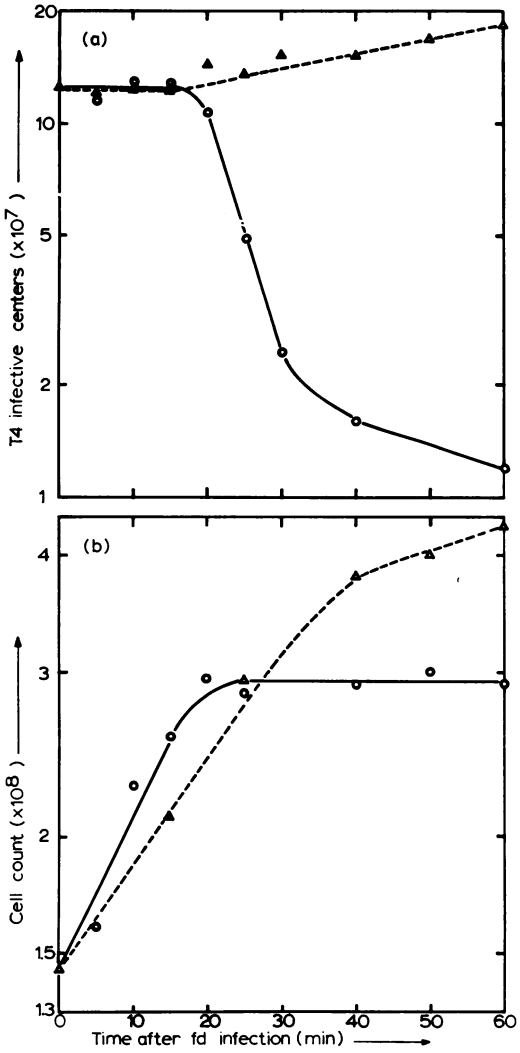

FIG. 18. *Killing by fd amber mutants. A culture of E. coli Hfr 2340 was grown to 3 × 10⁸/ml, divided, and infected at a multiplicity of 100 with fd wild type (△) and fd amber strain 9 (○). At various times, portions were removed and superinfected with T4 at a multiplicity of 2.5. After 5 min of adsorption, the bacteria were diluted through T4 antiserum and plated on E. coli K-12 P678 (F⁻). (a) T4 infective centers. (b) Number of bacterial cells by Petroff-Hausser counter. (From reference 91.)*

of the latent period (Fig. 10). Such a decrease in DNA synthesis is observed with mutants in genes 1, 3, 4, 5, or 6. Cells infected with gene 2 mutants under nonpermissive conditions incorporate thymidine pulses in the same way as uninfected cells.

Incorporation of 45-sec, ^{3}H-uridine pulses also drops off sharply at the end of the latent period (Fig. 16). Presumably, these short pulses measure primarily mRNA synthesis.

Protein synthesis, as measured by incorporation of ^{3}H-amino acids, decreases at the end of the latent period (Fig. 17). However, induction of β-galactosidase starts to fall from the moment of infection; in normal infection, the curve rises again after 15 min, but in killed cells the curve continues down (Fig. 17).

These changes have been compared (91) to the changes induced by the action of colicins on sensitive bacteria (137). Like many colicins, killing mutants stop DNA, RNA, and protein synthesis. Like colicins, killing mutants carry their genetic

TABLE 11. *Single-cell analysis of cells growing in antiserum against fd*[a]

No. of colonies (*n*)	No. of plates with *n* colonies	
	Infected culture	Uninfected culture
0	113	79
1	31	1
2	3	4
3	0	1
4	0	6
5	0	2
6–30	3	58

[a] Bacteria strain H12R8a (derivative of *E. coli* HfrC, from A. Garen) was infected with fd and plated; a single infected colony was picked and regrown in parallel with an uninfected culture in tryptone medium. At 6 × 10⁷ cells/ml, the cultures were diluted to about 600 cells/ml and 0.1 ml was plated for colony formers; the cultures were then further diluted to about 2.5 cells/ml in fd antiserum (preadsorbed with Hfr bacteria; $K = 2$ min^{-1}) at 37 C. After 20 min, two drops (0.12 ml) of the infected culture were added to each of 150 tubes, the tubes were incubated for 90 min at 37 C, and the contents of each tube were plated for colony formers. A parallel procedure was used for the uninfected culture. For the uninfected culture, we calculated 0.65 bacterium per tube from the fraction of tubes showing no colonies, compared with 0.30 calculated from the number of colony formers present before antiserum was added. Thus, the cells divided in antiserum. The two numbers are the same (0.28) for infected cells. Most uninfected tubes which did give colonies gave about 20 colonies, as expected for a doubling time of 20 min. Most infected tubes gave only one colony; thus, the bacterium in the tube did not divide in antiserum. Another control experiment, with infected bacteria and serum taken from the same rabbit before fd was injected, gave results as with uninfected bacteria; the effect is specific for antibodies against fd. See reference 63 for a discussion of the technique of single-cell analysis.

information on an extrachromosomal plasmid. However, unlike the action of colicins, killing takes place within the cell harboring the plasmid.

The simplest statement of the circumstances leading to killing is that gene 2 starts some potentially lethal chain of events which is normally stopped with the release of progeny virus. The gene 2 product may be a membrane-bound protein, and killing may act on membrane structure, as suggested for colicins (137). Changes in membrane morphology are observed in infected cells (24), especially under conditions which lead to killing (165).

Antiserum Effect

When antiserum against Ff is added to a culture of bacteria infected with the virus, the division of infected cells stops. Uninfected cells continue to grow and can quickly take over the culture. Some virus strains which have a defective but still functional gene 2 do not show this effect; that is, cells infected with these strains divide as well as uninfected cells in the presence of antiserum (94; Table 11).

These experiments suggest that one of the effects of gene 2 is on the bacterial envelope. Comparison with ϕX174 suggests that the gene 2 product, which promotes RF replication, is bound to the cell membrane. If the gene 2 product is attached to the membrane at a point of junction between the inner and outer parts of the cell envelope (14, 15), the same protein could affect not only RF replication and killing (internal functions) but also the antiserum effect (external function).

MODEL OF THE MOLECULAR PHYSIOLOGY OF FV

In this section, we bring together the various models which we have proposed to explain the present data on FV. Like all working hypotheses, this is our current best guess at the truth, but revision of the general model will certainly be required as more experiments are done. For convenience, we will use the indicative rather than the conditional mood in this section, but this choice of style should not be interpreted to mean that any statements in this section are necessarily true.

The virion is a ring of single-stranded DNA wrapped in a tube of α-helical B protein molecules; the whole nucleoprotein ring is formed into a linear molecule like a circle of string pulled taut from opposite sides of its circumference. The A protein is located at one end of the linear structure.

The virion diffuses into the neighborhood of the tip of the pilus, and the A protein attaches firmly to the end of the pilus. The attachment between A protein and pilus protein causes a structural change in the latter, which is transmitted from one subunit to the next of the pilus, down to the base of the pilus, where the altered pilus configuration induces a disaggregation of the pilus from the base within the cell. As the base of the pilus disaggregates, the pilus polymer is retracted back into the cell, pulling the attached virus with it.

As the virus passes through the membrane, the protein dissolves in the membrane. The DNA passes into the cell, where it is converted to RF by cell enzymes. The RF is transcribed to mRNA, which is expressed as viral proteins. Virus infection alters some of the translational machinery so that it is preempted for viral messenger.

An early protein to be made is the product of gene 2 expression. This protein is a bifunctional site for RF replication, which is bound to the cell membrane.

One of the functions of the gene 2 product is stimulation of the DNA synthesis involved in RF replication. The protein is not a DNA polymerase but has a secondary, structural role. If this gene 2 function is absent, RF is not replicated. If it is defective, as in the VP mutant of HR, or in the metavirus state, RF is replicated very slowly.

The second function of the gene 2 product is regulatory. When this site binds to cell membrane, it alters the cell membrane so as to prevent other sites from becoming functional in the neighborhood of the existing site (the site-territory). Cells growing in broth are large enough so that several virus site-territories may coexist with host chromosome site-territories. Clear plaque mutants of the virus have an altered gene 2 product which creates a larger site-territory, thereby inhibiting the formation of host chromosome sites and reducing the frequency of cell division.

Killing of host cells and the antiserum effect both act via the gene 2 product to drastically alter the cell membrane and turn off host functions.

Single-stranded viral DNA is produced from the beginning of stage II, by copying from the complementary strand of the URF. But this VS is immediately converted to RF by host enzymes. At the end of stage II, these host enzymes are blocked by the gene 5 product, so that free VS start to appear. This scheme gives an exponential increase of RF during stage II, even though only one RF replicates semiconservatively.

The B protein is stored in the membrane as fast as it is made. The protein forms a single tract of α helix, 7.5 nm in length, with charged groups at each end and a hydrophobic region in the middle. This molecule is stored in the membrane with the axis of the α helix perpendicular to the membrane

surface. [Membranes are 7.5 nm thick and consist of a hydrophobic region sandwiched between two hydrophilic regions; membrane proteins have a large α-helix content (110, 193)].

At the end of the latent period, the single-stranded viral DNA passes out through the membrane, picking up B protein as it goes.

The site of action of the antiserum effect is either the B protein which is stored in the membrane or the partially formed virions which are extruding from the membrane. The altered membrane which results from the reaction of B protein with antiviral serum interacts with the gene 2 product to inhibit cell functions.

Introduction of foreign proteins into cell membrane has been proposed as a common feature of cancer cells (16, 20, 192). The hypothesis of changed properties of bacteria as a result of introduction of FV protein into bacterial membrane would parallel this hypothesis. Although the analogy perhaps should not be pushed, it is worth keeping in mind that study of FV might well give us information useful in the medically more interesting, but technically more complex, case of viruses which cause cancer.

APPENDIX

Classification

The classification of viruses is a topic of debate (5, 71, 72, 120, 121). The most useful criterion for classification is morphology: type of nucleic acid, general shape [isometric or anisometric (86)], detailed shape (121). Of course, this criterion classifies only the extracellular infective particle or virion. An additional criterion is adsorption to host (host range). This is effectively a minor morphological criterion, since a different adsorption range presumably indicates a different adsorption protein. By this criterion, all F-specific viruses are related and all I-specific viruses are related.

The amino acid composition of the major coat protein (B protein) is a useful indication of relationship; fd and M-13 are identical in this respect, and f1 and ZJ/2 are not very different (Table 6). On the other hand, there are several marked differences between the amino acid compositions of If1 and fd (Wiseman and Marvin, *in preparation*). Serological relationships (Table 7) are somewhat difficult to interpret. Indirect experiments suggest that the inactivation of plaque-forming ability depends on a reaction along the side of the virion (168); however, electron micrographs show that If antiserum reacts along the side of fd and Ec9, even though the plaque-forming ability of these viruses is not affected by If antiserum (129). Inactivation of plaque-forming ability probably does not depend solely on the reaction of antiserum with the B protein, since fd and M-13 have the same B protein but different serological behavior. A mutant which is thought to have an altered B protein shows altered sensitivity to antiserum (144). Neither antigenic relationship nor host-controlled modification is a particularly stringent criterion of relationship, since significant changes in these properties can be induced by a point mutation (144; Table 10).

Another criterion of relationship is genetic relationship, the ability of mutants in different viruses to complement each other. M-13 has been shown to be related by this criterion to fd, f1, and HR. It need not be true that all filamentous bacterial viruses are genetically closely related, since similarity in structure may reflect not a common ancestry but an optimal design.

In the Lwoff-Horne-Tournier system, fd is classified as subphylum *Deoxyvira*, class *Deoxyhelica*, family *Inophagoviridae*, genus *Inophagovirus*, species *Inophagovirus bacterii* (121). "*Phagovirus*" is redundant, and we suggest altering the name of the family to *Inoviridae* and the type genus to *Inovirus*. We define the family *Inoviridae* to include all filamentous viruses which appear to be 5.5 $\pm$ 1.5 nm in diameter in the electron microscope, have a buoyant density of 1.28 $\pm$ 0.05 g/cm^3 in CsCl, and contain single-stranded circular DNA. The range of diameters reflects the variation in electron microscope measurements reported for viruses of the same real diameter. The final criterion for inclusion in this family is that the X-ray fiber diffraction pattern shows the same overall intensity distribution as that of fd (Fig. 7), especially the strong 1.0-nm equatorial reflection. The X-ray diffraction criterion is effectively a definition of morphology independent of length, since similar structures give similar fiber diffraction patterns, but dissimilar structures do not give similar diffraction patterns. The viruses fd, f1, M-13, and If1 have been classified as *Inoviridae* by this criterion (123, 130; Fig. 7).

Two length classes have been observed, one about 870 nm and one about 1,300 nm (Table 4). We define the type genus *Inovirus* to include all *Inoviridae* with a length of 870 $\pm$ 217.5 nm, and the genus *Dolichoinovirus* to include all *Inoviridae* with a length of 1,305 $\pm$ 217.5 nm. The lengths are chosen to make *Dolichoinovirus* exactly 1.5 times as long as *Inovirus*. The ranges are chosen to include lengths between these two standard lengths but not lengths far outside them.

Rather than use the species name (*bacterii*) proposed by Lwoff and Tournier (121), we define the species according to the host range (a morphological criterion). We propose the species

name *andreios* for viruses which adsorb to F pili, so that fd is *Inovirus andreios*, and is the type species of the genus *Inovirus*. We propose the species name *colicinus* for viruses which adsorb to I pili, so that If1 is *Dolichoinovirus colicinus* and is the type species of the genus *Dolichoinovirus*.

Whether all filamentous viruses adsorb to sex pili is unclear, since, in the four cases left unclassified in Table 1, the question has not been sufficiently investigated. An attempt to show that Pf1 is specific for known male strains of *P. aeruginosa* was unsuccessful, and electron micrographs of the host for Pf1 failed to reveal pili. On the other hand, the host for Pf1 is apparently able to transfer genetic markers to female strains of *P. aeruginosa*, so that it may be assumed to harbor a sex factor and thus to form sex pili (132).

In the vernacular name and cryptogram system (72), the cryptogram for fd is **D/1:2/12:U/U:B/0**, and the cryptogram for If1 is **D/1:3/12:U/U:B/0**. For convenience, we define vernacular names corresponding to the Lwoff-Horne-Tournier classifications discussed above. Members of the family *Inoviridae* are referred to as FV. Members of the species *I. andreios* are referred to as Ff. Members of the species. *D. colicinus* are referred to as If.

Glossary

The single-letter abbreviations for nucleosides and the three-letter abbreviations for amino acids of the IUPAC-IUB Commission on Biochemical Nomenclature are used without further definition.

We attempted to follow existing proposals for nomenclature in bacterial genetics (56, 184). But for suppressor strains of bacteria, we used a more customary system (70): *Su-1*, not *SupD*; *Su-2*, not *SupE*; *Su-3*, not *SupF*; *Su-4*, not *SupC*; and *Su-5*, not *SupG*.

In addition, the following abbreviations were used in this paper.

AMU. Atomic mass unit (1.66×10^{-24} g).

A protein. The adsorption protein, or assembly protein, located at one end of the virion.

B protein. The bulk protein, or major coat protein, of the virion.

BU. 5-Bromouracil.

CM. Chloramphenicol.

CS. Complementary strand. The DNA strand with base sequence complementary to viral DNA. It may be present as a single strand, but more frequently it is present as half of a double-stranded replicative form. It may be linear or circular and may be distinguished from VS in an alkaline CsCl equilibrium gradient, where it has a buoyant density of 1.748 g/cm³ compared with 1.763 g/cm³ for VS (152).

Ff. F-specific filamentous bacterial virus, *I. andreios*. A filamentous virus which adsorbs to F pili and has a length of about 870 nm.

FV. Filamentous virus, a member of the family *Inoviridae*. Any virus containing single-stranded circular DNA with a structure along the shaft similar to fd structure.

If. I-specific filamentous bacterial virus, *D. colicinus*. A filamentous virus which adsorbs to I pili and has a length of about 1,300 nm.

MC. Mitomycin C.

mRNA. Messenger RNA.

RF. Replicative form. The double-stranded intracellular form of FV DNA. Both strands may be circular or either strand broken. It has a buoyant density of 1.701 g/cm³ in neutral CsCl, relative to *E. coli* at 1.710 g/cm³ (154).

RF I. RF with both strands circular. Sediments with 24*S* at neutral *p*H (154).

RF II. RF with one strand nicked. Sediments with 19*S* at neutral *p*H (154).

SDS. Sodium dodecyl sulfate.

URF. Unique replicative form. The replicative form which consists of an infecting parental VS plus the first CS to be made.

VS. Viral strand. The DNA extracted from the virion or the homologous intracellular form. It may be linear or circular.

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